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Effect of an improved complementary food on nutrition of Zambian infants

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Abstract

Background: Infant growth faltering is common in developing countries. A major cause is inappropriate feeding practices and poor quality complementary foods with low energy density and deficiency in micronutrients and which may displace breast milk. The impact of micronutrient fortification of complementary foods or adding α-amylase to increase energy density on growth of infants from middle income urban populations in developing countries has been inadequately studied.

Objectives: To assess current complementary feeding practices, develop and test the acceptability and effect of amylase-treated-fortified complementary blends named Chilenje Baby Mix (CBM) on growth and haemoglobin concentration of infants from a middle income community in Lusaka, Zambia.

Design: Three-stage study comprising assessment of, 1) current complementary feeding practices, 2) acceptability of amylase-treated-fortified complementary food and, 3) the effect of the complementary food on growth, haemoglobin concentration and breast milk intake of 9-month old infants in a randomized controlled trial.

Methods: Current complementary feeding practices were assessed by qualitative (focus group discussions, interviews and home observations) and quantitative methods. Acceptability to mothers of roasted maize-beans-groundnuts-bambaranuts porridge recipes was assessed prior to the industrial production of α-amylase-treated-fortified blends.

Infants were randomized to receive a fortified blend with (CBMA) or without amylase (CBM) from 6 – 9 months of age. Anthropometric measurements, dietary intake and morbidity were determined monthly. Non-intervention infants were

measured at 9 months of age. Breast milk intake was determined by deuterium oxide dilution method in a non-random subset of infants at 9 months.

Results: Mothers had wide knowledge on optimal infant feeding, but actual practices were constrained by food cost and time availability. α-amylase enhanced porridge acceptability and caused 1000-fold reduction in porridge viscosity. The developed blend cost half as much as the average price of commercially complementary foods in the market.

Mean weight gain between 6 and 9 months was 1.0 (SD 0.6) kg, 0.9 (SD 0.6) kg and 0.9 (SD 0.5) kg for infants in the CBM, CBMA and control groups, respectively ($p = 0.54$). Mean length at 9 months was 71.8 (SD 2.5) cm, 71.3 (SD 1.5) cm and 70.9 (SD 2.4) cm for infants in the CBM, CBMA and control groups, respectively ($p = 0.06$); infants in CBM had significantly greater length ($p = 0.04$) than infants in the control group by least square differences. Infants in both CBM and CBMA had significantly greater biceps ($p = 0.02$), subscapular ($p < 0.001$) and suprailiac skinfolds (< 0.001) and percent fat mass ($p = 0.01$) than infants in the control group. Infants in both CBM and CBMA had significantly greater haemoglobin concentration ($p = 0.03$) than infants in the control group [104 (SD 12) g/L, 103 (SD 12) g/L and 98 (SD 14) g/L for CBM, CBMA and control groups, respectively]. The mean breast milk intake was 614 (SD 271) g/day, 635 (SD 193) g/day and 653 (SD 221) g/day ($p = 0.87$) and the mean energy intakes from breast milk and complementary foods represented 116%, 112% and 115% of the RDA for infants in CBM, CBMA and control groups, respectively. Infants in both CBM and CBMA had significantly greater intakes of calcium ($p = 0.01$), iron ($p < 0.001$), zinc ($p < 0.001$), vitamin A ($p = 0.01$), vitamin C ($p < 0.001$), thiamine ($p = 0.03$) and riboflavin ($p = 0.02$) than infants in the control group.

Conclusion

A cheap and acceptable industrially processed fortified complementary blend was developed. The porridge blend resulted in moderate improvement in infant length, but had more obvious improvement in haemoglobin concentration without adverse effect on breast milk intake. This blend may be used to improve micronutrient status and health of infants in middle income urban communities in developing countries.

Dedication

To the memory of my late parents, Margaret Agolla Owino and Owino Ndian'g who gave me identity and the best nutrition they could afford in their circumstances.

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"He giveth power to the faint; and to them that have no might he increaseth strength. Even the youths shall faint and be weary, and the young men shall utterly fall; but they that wait upon the LORD shall renew their strength; they shall mount up with wings as eagles; they shall run, and not be weary; and they shall walk, and not faint". (Isaiah 40: 29-31).

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Abbreviations

AFB_1: Aflatoxin B_1

AFB_2: Aflatoxin B_2

AFG_1: Aflatoxin G_1

AFG_2: Aflatoxin G_2

ANOVA: Analysis of Varaince

ARF: Amylase Rich Flour

AOAC: Association of Official Analytical Chemists.

ARI: Acute Respiratory Infection

BMI: Body Mass Index

CBM: Chilenje Baby Mix

CBMA: Chilenje Baby Mix with Amylase

CIGNIS: Chilenje Infant Growth Nutrition and Infection Study

CSB: Corn-Soy Blend

DPT: Diptheria, Pertussis, Tetanus.

EC: Escherichia coli

EDTA: Ethyldiamine trichloroacetic acid

FAO: Food and Agricultural Organization

FFQs: Food Frequency Questionnaires

FGD: Focus Group Discussion

GOSH: The Great Ormond Street Hospital

HAZ: Length-for-Age

HEPS: High Energy Protein Supplement

HIV: Human Immunodeficiency virus

HPLC: High Performance Liquid Chromatography

IAEA: International Atomic Energy Agency

ICH: Institute of Child Health

ID: Identification

IgA: Immunoglobulin A

ILSI: Interantional Life Science Institute

IVACG: International Vitamin A Consultative Group

IWGFNC: Informal Working Group on Feeding of Nonbreastfed Children

IZiNCG: International Zinc Nutrition Consultative Group

LST: Lauryl Sulphate Tryptose

MAMA: Mid Arm Muscle Area

MAFA: Mid Arm Fat Area

MPN: Most Probable Number

MRC: Medical Research Council

MTCT: Mother-to-child-transmission (of HIV)

MUAC: Mid Upper Arm Circumference

NCHS: National Centre for Health Statistics

NGO: Non Governmental Organization

NPU: Net Protein Untilization

PER: Protein Efficiency Ration

RDA: Recommended Dietary Allowance

RDI: Recommended Dietary Intake

RNI: Recommended Nutrient Intake

RPHPLC: Reversed Phase High Performance Liquid Chromatography

SPSS: Statistical Package for the Social Sciences

TB: Tuberculosis

TBW: Total Body Water

TM: Trade Mark

TW: Tryptone Water

UNICEF: United Nations Children's Fund

UK: United Kingdom

VO: Victor Owino

VSMOW: Vienna Standard Mean Ocean Water

WAZ: Weight-for-Age

WFP: World Food Programme

WHO: World Health Organization

WHZ: Weight-for-Length

WSB: Wheat-Soy Blend

1 Introduction

Growth faltering marked by increased stunting from birth and underweight and wasting from 3 months of age, persisting for at least the first 18-24 months of life (Shrimpton et al, 2001), is a major public problem in developing countries. The rapid acceleration of growth faltering occurring between 6 -12 months of life (Shrimpton et al, 2001) coincides with the introduction of complementary foods when breast milk alone is not sufficient to meet entire nutritional requirements of infants (WHO, 2002; Gibson et al, 1998).

Complementary foods in developing countries often fail to meet the nutritional requirements of infants because they are mostly cereal-le.g.ume based and have low energy and nutrient density and contain inhibitory ligands such as phytic acid that limit the absorption of essential micronutrients such as iron and zinc (Gibson et al, 1998). The problem is particularly serious for infants who may not be breastfed for reasons including maternal HIV-infection, death or severe illness of the mother or lack of the desire for the mother to breastfeed (Dewey et al, 2004). Micronutrient deficiencies, especially those of iron, zinc and calcium are likely to occur in these circumstances (Dewey, 2000).

Several recommendations including the wide use of indigenous foodstuffs and industrial processing procedures (WHO, 2002; Dewey and Brown, 2003) for the improvement of the quality of complementary foods have been proposed. Food fortification may be used increase micronutrient density. Energy density may be improved by the addition of oil and sugar (Dewey and Brown, 2003) or by the reduction of the amount of water added during complementary food preparation.

Alternatively, enzymes such as α-amylase which break down starch, thereby enabling the addition of more dry flour per unit amount of water, may be used to increase food intake (Dewey and Brown, 2003; Darnton-Hill and Nalubola, 2002; Mannar and Sankar, 2004) and industrial processing techniques such as extrusion cooking (Dewey and Brown, 2003) to ensure good hygiene. Even though many programmes aimed at improving complementary feeding have been undertaken in the developing world (Piwoz et al 2003) there is inadequate data on energy and nutrient intake from complementary foods. Dietary intake data can be used to determine the level of nutrient addition during fortification of complementary foods. Such data can also be the basis for the recommendation of daily rations (Lutter, 2003) and for the education of mothers and caretakers on sound feeding practices that can help prevent early growth faltering.

Complementary foods may partially displace breast milk (Haisma et al, 2003; Haisma et al, 2004) and may also interfere with the absorption of nutrients in breast milk (Brown et al, 1995) thereby producing a greater likelihood of nutrient deficiencies. Knowledge of breast milk intake by infants is required for the estimation of daily nutrient intake during this period of an infant's life (Hendrickse and Wamberg, 1999). The dearth of published breast milk intake data (Dewey and Brown, 2003), particularly for older infants (6 – 12 months old), in low-income countries has been underscored.

Previous studies assessing the effect of improved complementary foods on growth and micronutrient status of infants have been carried out among disadvantaged (rural, acutely ill, or severely malnourished infants and young children and their

results on growth and micronutrient status have been mixed (Dewey, 2000) and further work has been recommended (Dewey, 2005). The dearth of data on the effect of improved complementary foods on growth of infants in relatively well-off populations has been underscored (Dewey, 2000). Iron deficiency is common in both developing and developed countries (Hascke, 1999) and may affect a much larger proportion of the population than those who have evident clinical symptoms (Maberly et al., 1998). The aim of this study was to assess the effect of a centrally processed, extrusion cooked, α-amylase-treated, multi-micronutrient fortified maize-bean-groundnut-bamabaranut complementary blend on growth, body composition and haemoglobin concentration and to generate data on the breast milk intake of 9-month old infants living a middle income, HIV-endemic urban community in Lusaka, Zambia.

2 Literature review

2.1 Malnutrition and child growth in developing countries

Malnutrition is one of the major public health problems in less developed countries. 70% of all childhood mortality and malnutrition are reported from sub-Saharan Africa and South Asia (Shrimpton et al. 2001). Poor nutrition leads to ill health which in turn leads to further deterioration in nutritional status, more so in infants and young children (WHO, 2003a). It is estimated that 33% and 27% of the world's children are stunted and underweight, respectively, a fact that accounts for over 50% of the annual deaths of children under 5 years (Black et al, 2003). The effects of poor nutrition and stunting continue over the child's life, contributing to poor school performance, reduced productivity, and other measures of impaired intellectual and social development (WHO, 2003a).

Malnutrition involves deficiencies not only of macronutrients, but also of micronutrients (Umeta et al., 2003). Three underlying causes of undernutrition that exist at household or family level are recognized, namely, 1) insufficient access to food; 2) poor health environment, i.e. poor water, deficient sanitation and inadequate health services and 3) care, i.e. inadequate maternal and child care practices (Martorell, 1999). These underlying casual factors lead to under-nutrition through two immediate mechanisms at the individual level, namely, inadequate dietary intakes and disease (Martorell, 1999).

Poor maternal nutrition, inappropriate breastfeeding and complementary feeding practices (WHO, 2003a) contribute to child malnutrition. Inappropriate feeding practices are a major cause of the onset of malnutrition in young children (WHO, 2003b). Family and caregiver characteristics, particularly education and household management or coping skills of the mother can determine growth in circumstances where economic resources are adequate (Pelto, 2000).

Growth assessment gives the best indication of the health and nutritional status of children because disturbances in health and nutrition, regardless of their etiology, affect child growth. Growth measurement also provides an indirect measurement of the quality of life of an entire population (de Onis et al, 1993). The assessment of malnutrition at population level is based on the measurement of anthropometric indicators such as weight and length, which are in turn used to construct indices that help in the interpretation of body measurements. Weight-for-height, height-for-age and weight-for-age are most commonly used indicators in children (de Onis and Blossner, 2003). Taking age and sex into account, differences in these measurements can be expressed as Z-scores (standard deviation units), or mean percentiles which enable comparison of a child or a group of children with a reference population (de Onis, 2000; de Onis and Blossner, 2003; Cogill, 2003). Although the National Centre for Health Statistics (NCHS) growth reference (NCHS/WHO international reference population) has been used to compare growth across countries since 1970s (de Onis and Blossner, 2003), it is considered inadequate for assessing the growth of breast-fed infants (de Onis and Habitch, 1996).

The development of a new multi-country international growth reference (de Onis, 2000) has been underway following previous recommendations (Victora et al, 1998).

Z-score unit, defined as the difference between the value for an individual and the median value of the reference population for the same age or height/length, divided by the standard deviation of the reference population, is more commonly used internationally for two main reasons. Firstly, it allows for identification of a fixed point in the distribution of different indices and across different ages. Secondly, the use of Z-scores makes it possible to calculate summary statistics (Cogill, 2003) such as means, standard deviation, standard error and frequency distribution (WHO, 2005). The other way of expressing Z-score results is by cut-off that enables the conversion of individual measurements to prevalence statistics (WHO, 2005, Cogill, 2003). The WHO Global Database on Child Growth and Malnutrition uses a Z-score cut-off point of <-2 SD to classify low weight-for-age, low height-for-age and low weight-for-height as moderate and severe undernutrition, and <-3 SD to define severe undernutrition. The cut-off point of >+2 SD classifies high weight-for-height as overweight in children (WHO, 2005). The most commonly used Z-score cut-off is -2 standard deviations irrespective of the indicator used. Low weight-for-age (underweight) reflects both chronic and acute malnutrition; low height-for-age (stunting) identifies chronic malnutrition; low weight-for-height (wasting) shows current or acute malnutrition (Cogill, 2003).

2.1.1 Protein energy malnutrition

Protein deficiency was perceived as the most important nutritional problem in developing countries three decades ago (Briend, 2005). A review of studies on complementary foods in developing countries (Dewey and Brown, 2003) showed that the protein density (2.0 to 3.3 g/100 kcal) was greater than the required level (0.7 to1.0 g/100 kcal). Although research on the causes of growth failure over the five decades focused on protein and then energy intake, there has been a shift and the focus is on ensuring micronutrient needs are met (Dewey and Brown, 2003; Ramakrishman et al, 2004). However, energy deficiency is still a major problem in developing countries.

The current recommendations for energy intake from complementary foods are 200 kcal/day and 300 kcal/d for infants 6 - 8 months and 9 - 11 months old, respectively, and 550 kcal/d for young children 12 - 24 months of age (Dewey and Brown, 2003). However, adequate energy intake is determined by two main properties of a given diet namely, food volume and energy density (Capdevila et al, 1998). It is difficult to achieve an adequate diet for children aged 6 - 24 months (Mensah and Tomkins, 2003) because most of the traditionally used complementary foods in developing countries are unfortified (Hotz and Gibson, 2001) cereal-based gruels characterized by high water content, and low energy and nutrient density (Gibson et al, 1998; den Besten et al, 1998).

The high volume/high-viscosity characteristic of cereal-based foods is commonly referred to as dietary bulk (Hansen et al, 1989). Most of the gruels prepared in households have an energy density of about 40 kcal/100 g (Vieu et al, 2001;

Chakravarthi and Kapoor, 2003) and may have to be fed frequently due to the limited gastric capacity of infants (Vieu et al, 2001). A previous study (Treche and Mbome, 1999) assessed the macronutrient content, viscosity and energy density of locally produced and imported commercial cereal-based flours. The workers found that the energy densities of 50% of the locally produced flours were too low to supply adequate energy to supplement breast milk even if the gruels were fed three times daily. A recent study (Mamiro et al, 2005) assessing feeding practices and factors contributing to growth faltering and iron deficiency in rural Tanzanian children 3-23 months old showed that children were mainly fed a thin porridge made from maize flour.

Although some studies (Khoshoo and Reifen, 2002; Traore et al, 2005) have demonstrated the benefit of improved energy density on infants energy intake, other evidence (Kimmons et al, 2005; Hotz and Gibson, 2005) shows that increased feeding frequency may be the most important factor. Several methods that can be used to enhance energy density of complementary foods include addition of oil (Bajaj et al, 2005) and digestion with α-amylase (Brown et al, 1998) to enable the addtition of more complementary food solids per unit amount of water thereby enhancing food intake. This thesis focuses on the application of α-amylase to improve complementary food intake and its effects on infant nutrient intake and growth which is discussed in detail later (see section 2.2.2.3.1).

2.1.2 Mineral and vitamin deficiencies

Micronutrients play important roles in the production of enzymes, hormones and other substances, helping to re.g.ulate growth, activity, development and the functioning of the immune and reproductive systems. Adequate intake of

micronutrients is especially crucial during early childhood and other periods of rapid growth, pregnancy and breastfeeding (UNICEF, 1998). The causes of micronutrient deficiencies include inadequate dietary intake, impaired absorption, limited bioavailability, excess losses, or a combination of these causes (ILSI, 1996). Micronutrients are divided into two groups (Table 2-1) based on the effect of maternal nutrient intake and status on breast milk content of a particular nutrient (WHO, 1998). However, emerging knowledge from research may result in re-classification of micronutrients. Nutrients such as folic acid may fall under intermediate classification.

The deficiencies of iron, iodine and vitamin A are the most common and of great concern for women and children in developing countries (UNICEF, 1998). Recently, iron, zinc, calcium, thiamine, riboflavin and pyridoxine have been identified as problem nutrients for infants and young children beyond 6 months of age in developing countries (Dewey and Brown, 2003). The need for multi-micronutrient interventions (supplementation or fortification) has been stressed since micronutrient deficiencies occur together, especially in Africa where diets are likely to be deficient in several nutrients (Piwoz and Prebble, 2000). Micronutrient interactions affect the bioavailability of micronutrients (ILSI, 1996) given as supplements or in fortified foods.

Table 2-1 Micronutrient cate.g.ories based on the effect of maternal intake and status on breast content of a particular nutrient[1,2]

Group I Affected by maternal status	Group II Not affected by maternal status
Thiamine	Folic acid
Riboflavin	Vitamin D
Pyridoxine	Calcium
Vitamin A	Iron
Iodine	Copper
Selenium	Zinc

[1]taken from WHO, 1998

[2]continuing research may result in re-classification of nutrients. Nutrients such as folic acid may fall under intermediate classification.

2.1.2.1 Iron deficiency

Iron is not only required to make haemoglobin concentration for red blood cells and for oxygen circulation in the body, but also for energy metabolism (Piwoz and Prebble, 2000). Iron deficiency anaemia is the most common nutritional disorder in the world (UNICEF, 1998). It not only affects all age groups of the under privile.g.ed population in most developing countries, but iron deficiency is also common among vulnerable groups such as adolescent girls and the elderly in affluent families (Bhaskaram, 2001). Iron deficiency anemia has adverse effects on mental, motor and emotional development in children and later cognitive performance (Lynch and Stoltzfus, 2003). It has been shown that the prevalence of iron deficiency anemia (haemoglobin concentration < 110 g/L and ferritin < 12

μg/L) is higher in HIV-infected than uninfected infants (Totin et al, 2002) and this is excerbated by malaria infection (Crawley, 2004). The causes of iron deficiency anemia in children include inadequate dietary intake and parasitic infections such as hookworm (UNICEF, 1998).

Iron reserves at birth and the small amount of highly bioavailable iron in breast milk (0.35 mg iron/ L) are adequate to meet the iron requirements of normal birth weight breast-fed infants for the first six months of life (Lynch and Stoltzfus, 2003). An increased requirement for iron occurs between 4 and 12 months of life, a need that cannot be met by breast milk alone (Krebs, 2000). To meet the iron needs of infants and young children, it is now recommended that 80% of the child's requirement for absorbable iron, 0.58 mg/d for infants aged 7 to 12 months and 0.54 mg/d for children aged 13 to 24 months, be supplied by complementary foods, iron supplements or both (Lynch and Stoltzfus, 2003). However, in developing countries iron deficiency is excerbated by the fact that complementary foods are based on mixtures of cereals and le.g.umes that are rich in iron chelating ligands such as phytic acid (Hurrell, 2003) and tannins.

2.1.2.2 Zinc deficiency

Zinc promotes normal growth and development and is an element in enzymes that work with red blood cells which move carbon dioxide from tissues to lungs (UNICEF, 1998). Clinical symptoms of marginal zinc deficiency are depressed immunity, impaired taste and smell, impaired memory and decreased spermatogenesis (Rosado, 2003). Single zinc supplementation during infancy has been found to result in significantly improved growth (Lind et al, 2004). Zinc, copper and selenium are linked together in cytosolic defence against reactive

oxygen and nitrogen species (Klotz et al, 2003). The deficiencies of these micronutrients may contribute to the pathogenesis of HIV infection through increased oxidative stress and compromised immunity. Milk zinc concentrations are quite high in the early weeks postpartum, averaging >3 mg/L at 2 wk, but then decline sharply over the early weeks of lactation, resulting in a longitudinal decline in zinc intake (Krebs, 2000).

2.1.2.3 Calcium deficiency

Calcium plays a role in neuro-transmission, muscle contraction, blood coagulation and skeletal support (Miller et al, 2001). The estimated total calcium intake from breast milk is 130 mg/d in infants aged 7-12 months (Abrams and Atkinson, 2003). Severe bone mineral deficiency in infants and young children is associated with nutritional rickets (Abrams and Atkinson, 2003). Vitamin D deficiency is the main cause of rickets in young infants (Pettifor, 2004), however, very low calcium intakes may be partially or primarily responsible for the disease in some children (Abrams and Atkinson, 2003). Daily calcium intakes less than 300 mg may pose a risk for the development of rickets notwithstanding normal vitamin D status (Abrams and Atkinson, 2004). Low calcium intakes in older infants and young children can be attributed, in most cases, to the consumption of cereal-based diets with little variety, high in phytate and less access to dairy products (Pettifor, 2004). In general populations, adequate calcium intake has been associated with reduced risk for chronic diseases such as osteoporosis and hypertension (Miller et al, 2001). Calcium can be obtained from foods naturally rich in calcium such as milk and milk products, calcium-fortified foods, from supplements or a combination of these sources (Miller et al, 2001).

2.1.2.4 Vitamin A deficiency

Vitamin A, one of the most important micronutrients affecting the health of children, is necessary for orderly growth and differentiation of tissues (Mactier and Weaver, 2005), and is essential for normal immune function (Semba et al, 2005). Vitamin A deficiency is the primary cause of blindness in children in developing countries and increases the risk of death from diarrhea, measles and malaria (Aguayo et al, 2005). An estimated 26-30% of the 127 million pre-school children estimated to be vitamin A deficient worldwide live in Sub-Saharan Africa. Vitamin A deficiency occurs primarily due to inadequate dietary intake of vitamin A. The estimated vitamin A gaps for breast-fed infants 6-11 months and breast-fed young children are 63-92 μg RE (16-23% of recommended daily intake) and 125 μg RE (31% of RDI), respectively (Mora, 2003).

The control of vitamin A deficiency is recognized to be a low-cost/high-impact intervention for child survival (Aguayo et al, 2005). Interventions to alleviate vitamin A deficiency include periodic pharmaceutical supplementation, food fortification and dietary diversification to increase consumption of vitamin A rich foods (Mora, 2003). Mandatory fortification of sugar with vitamin A has been implemented in several developing countries including El Salvador, Guatemala, Honduras, Nicaragua (Mora, 2003) and Zambia (Serlemistsos and Fusco, 2001).

2.1.2.5 Deficiency of B-vitamins

While there is a wealth of knowledge on the prevalence and consequences of iron, vitamin A and iodine deficiencies, there seems to be lack of similar knowledge with re.g.ard to most of the B-vitamins (Bhan et al, 2001; Ramakrishan, 2002). Low maternal intakes or stores during lactation of B-vitamins, except for folate,

result in reduced breast milk concentration (WHO, 1998) as shown in Table 2-1 above. Further, infants' stores are readily depleted, hence increasing the child's dependence on adequate amounts in complementary foods (Allen, 2003).

2.1.2.5.1 Thiamine

Thiamine helps to produce energy from carbohydrate and is important in maintaining skin and mucosal health (Shimizu, 2002). Thiamine deficiency in children leads to beriberi. The main symptoms of thiamine deficiency are peripheral neuropathy, encephalopathy and cardiac failure in infants (Allen, 2003).

2.1.2.5.2 Riboflavin

Riboflavin is a water-soluble vitamin present in a wide variety of foods including milk and milk products, meats, green leafy ve.g.etables and fruits. Its most important biologically active forms such as flavin adenine dinucleotide participate in a range of redox reactions, some of which are key to the function of aerobic cells (Powers, 2003). Riboflavin deficiency usually occurs in combination with other B-vitamin deficiency conditions such Pellagra (McCabe, 2001). Riboflavin deficiency can cause conditioned deficiency of pyridoxine thereby resulting in impaired skin collagen maturity and associated mucocutaneous lesions. Subclinical riboflavin deficiency impairs psychomotor function (Lakshmi, 1998). Riboflavin deficiency may also lead to increased oxidative stress due to restricted re.g.eneration of reduced glutathione (Das et al, 1990). Poor riboflavin status interferes with iron metabolism and contributes to the etiology of anemia when iron intakes are low (Powers, 2003).

2.1.2.5.3 Pyridoxine

Pyridoxine plays a major role in amino acid metabolism and the actions of steroid hormones. The greater part of the body's pyridoxine is in muscle, associated with glycogen phosphorylase (Bender, 1989). It is also important in lipid metabolism and the immune process (Serfotein et al, 1984). Pyridoxine is involved in the re.g.ulation of mental function and mood and is an essential homocysteine re-methylation cofactor. Its deficiency is associated with increased blood homocysteine levels, a risk factor for cerebrovascular disease (Malouf and Grimley, 2003). Pyridoxine deficiency leads to impairment of immune responses through the decreased rate of production of one-carbon units necessary for the synthesis of nucleic acids (Trakatellis et al, 1997).

2.1.2.6 Nutrient interactions

The importance of micronutrient interactions in determining nutrient biovailability (ILSI, 1996) has prompted comprehensive reviews on general micronutrient interactions (Sandstrom, 2001; Black, 2001) and interactions between specific micronutrients such as iron-zinc (Walker et al, 2005; Fairweather-Tait, 1995), iron-zinc-copper (Lonnerdal, 1996), iron-ascorbic acid and iron-calcium (Allen, 1996; Fairweather-Tait, 1995), vitamin A-iron (Schultink and Gross, 1996), riboflavin-iron (Powers, 1996). The influence of phytic acid on micronutrient interactions has also been reviewed (Davidsson, 1996). Micronutrients interact in two major ways (Lonnerdal, 1996) namely, 1) two (or more) elements share the same absorptive pathway, and 2) deficiency of one element affects the metabolism of another element.

In the first mechanism, an excess of one element can induce a deficiency of the other and has been demonstrated in iron-zinc-copper interactions (Lonnerdal, 1996, Sandstrom, 2001). Reviews on iron-zinc-copper interactions (Sandstrom, 2001; Walker et al, 2005; Lonnerdal, 1996, Fairweather-Tait, 1995) have shown the adverse effect of zinc on iron and copper status. Zinc supplementation in combination with iron, but not zinc alone appears to have ne.g.ative effect on iron status (Walker et al, 2005). Combined supply of vitamin A and iron as supplements or in fortified food may positively influence iron status (Schultink and Gross, 1996). The effect of iron on zinc status depends on iron:zinc ratio (Fairweather-Tait, 1995). Iron:zinc ratio of 25:1 in water solution, but not in meals (Lonnerdal, 1996) can result in measurable effect on human zinc status (Fairweather-Tait, 1995). Iron supplementation (Walker, 2005) or fortifying food with iron (Fairweather-Tait, 1995) do not have adverse effects on zinc absorption and status.

The absorption of non-heme iron is strongly influenced by the presence of ascorbic acid (Allen, 1996) in the same diet. This is either through the reduction of iron from less absorbed ferric to a more absorbed ferrous form or the formation of iron-ascorbic acid chelate hence making iron unavailable for chelation by inhibitory ligands such as phytic acid and tannins (Allen, 1996). A study in Jamaica (Davidsson et al, 1998) assessing the influence of ascorbic on iron absorption from an iron-fortified chocolate-flavoured milk drink in children 6-7 years old found lower iron absorption without ascorbic acid (1.6%), but this significantly improved to 5.1% ($p < 0.0001$) and 5.4% ($p < 0.05$) with 25 mg and 50 mg of ascorbic acid per 25 g serving, respectively. Although iron-ascorbic

molar ratios from 1:2 have been shown to improve iron absorption (Allen, 1996) it seems that the molar ratio is not the critical factor (Lynch and Stoltzfus, 2003) in iron-ascorbic acid interaction. It has been suggested that the absolute amount of ascorbic acid in the meal and the ratio between the concentration of ascorbic acid and inhibitory ligands, especially phytate, may be more important (Lynch and Stolzfus, 2003. The addition of ascorbic acid in high enough amounts can overcome the inhibitory effect of phytic acid from infant cereals with low phytic acid content (Davidsson, 1996). The current recommendations (Lynch and Stoltzfus, 2003) of ascorbic acid addition to iron-fortified foods for infants are based on ascorbic-iron molar ratio between 2:1 and 4:1 (70–140 mg/d).

Iron-calcium interactions depend on a number of factors including the amount of calcium, form of calcium, whether the micronutrients are obtained from food or not, timing of calcium administration and individual iron status (Allen, 1996). High amounts of calcium (> 600 mg/d) have lower inhibitory effect on iron absorption than lower calcium levels (about 300 mg/d) (Fairweather-Tait, 1995; Allen, 1996). This observation seems to be valid with high levels of phytate (> 400 mg/d) and the preferential binding of calcium by phytate which avails more iron for absorption (Fairweather-Tait, 1995). The influence of other factors on calcium-iron interaction has been summarized (Allen, 1996) It is apparent that soluble forms of calcium (citrate, citrate malate) have higher adverse effect on iron absorption. The inhibition of iron absorption is higher when calcium and iron are consumed with food, especially if the food contains iron of low bioavailability and when calcium is consumed close to the time of iron consumption. The effect of calcium on iron absorption is more pronounced in iron-depleted individuals.

Several examples have been reviewed under the second micronutrient interaction mechanism. Copper deficiency causes iron deficiency anemia by way of decreased ferroxidase (ceruloplasmin) activity thereby interfering with mobilization of iron from stores and its incorporation into haemoglobin concentration (Lonnerdal, 1996). Riboflavin deficiency can cause conditioned deficiency of thiamine (Lakshmi, 1998) and may contribute to the etiology of anemia when iron intakes are low (Powers, 2003). Vitamin A deficiency can cause iron-deficiency anemia (IVACG, 1998).

In summary, for nutrients that compete for absorption such as iron, zinc and calcium, the influence of naturally occurring nutrient inhibiting ligands such as phytic acid must be considered. This is relevant in developing countries where nutrient availability from complementary foods is low due to high levels of phytic acid. Food processing procedures that limit the adverse effects of phytic acid on nutrient absorption need to be considered. Phytic acid and the effect of extrusion cooking on phytic acid are described later in this thesis.

2.1.3 HIV/AIDS and infant and young child nutrition and growth

Growth failure in HIV-positive children is a common feature that can be used as a sensitive indicator of disease progression. Stunting accompanied by preferential decrease of lean body mass is the most common abnormality in HIV-infected children (Arpadi, 2000). A strong association between growth failure and increased mortality in HIV-infected infants has been shown (Berhane et al, 1997). HIV-infected infants are usually shorter and lighter than uninfected children at

birth and this trend remains several months after birth in both sexes (Moye et al, 1996). Several studies have shown no difference in growth pattern between HIV-uninfected infants born to HIV-seropositive mothers and non-HIV-exposed infants (Bailey et al, 1999; Newell et al, 2003; Lepage et al, 1996). However, uninfected infants of HIV-seropositive mothers tend to be lighter at birth than infants of HIV-uninfected mothers, but they catch up after three months and maintain the similar weight and length afterward (Bailey et al, 1999; Agostoni et al, 1998). This catch up growth has been attributed to fat deposition in a compensatory rebound mechanism upon nutritional intervention in a similar pattern observed in children with HIV infection (Agostoni et al, 1998). Effects of nutrition interventions on the body composition of HIV-exposed infants have not been assessed adequately and more work is warranted. It has been observed that HIV-infection and HIV-associated symptoms, but not maternal HIV status, are risk factors for growth retardation in children (Bailey et al, 1999). This underscores the importance of prevention of mother-to-child transmission of HIV. Mother-to-child transmission (MTCT) of HIV is the largest source of HIV infection in infant and children, accounting for more than 90% of the 2.2 million HIV-infected children living in Africa (Anabwani et al, 2005). The overall risk of MTCT after 6 months of age is estimated to be 9.3% (Coutsoudis et al, 2004). Vertical transmission of HIV may be prevented by the administration of highly active antiretroviral therapies to mothers during pre.g.nancy, labor, delivery and to infants post-natally (Connor et al, 1994). A recent decision analysis showed that antiretroviral therapy while still maintaining breastfeeding may prevent most childhood deaths associated with HIV than strate.g.ies involving early weaning and avoidance of breastfeeding (Bertolli et al, 2003). Perinatal administration of

nevirapine and zidovudine resulted in three-fold reductions (from 48% to 16.4%) in mother-to-child transmission of HIV in Uganda (Bajunirwe et al, 2005).

While most studies have shown the benefits of antiretroviral therapies in preventing vertical transmission of HIV (Kagaayi et al, 2005; Songok et al, 2003), improving child growth (Newell et al, 2003; Verweel et al, 2002) and reducing mortality (Dabis et al, 2001), it has been shown that these therapies have no effect on growth in uninfected European children born to HIV-positive mothers (Newell et al, 2003). This shows that antiretroviral therapy in combination with breastfeeding may be the recommended option for HIV-positive mothers whose infants are also HIV-infected. There are currently national and international initiatives to accelerate access of HIV-infected children to antiretroviral drugs in developing countries (Anabwani et al 2005). However, there is need for a different approach in cases where infants are not yet infected with HIV.

The high prevalence of HIV in developing countries has adversely affected recommendations for infant feeding. One-third to one half of mother-to-child transmission of HIV in developing countries is through breastfeeding (WHO, 2002). The knowledge by mothers of their HIV status has been shown to influence breastfeeding and complementary feeding practices and attitudes in developing countries (Omari et al 2003; Talawat et al. 2002, Chisenga et al, 2005). While HIV-infected mothers in developed countries are advised to avoid breastfeeding, the situation is more complicated in resource poor settings where there is need to strike a balance between the risk of postnatal HIV transmission through breast milk and the risk of morbidity and mortality associated with

replacement feeding (WHO, 2003b, Coutsoudis et al. 1999, Talawat et al. 2002). In resource poor settings, exclusive breastfeeding for the first 6 months is recommended except in situations where replacement feeding is acceptable, feasible, affordable, sustainable and safe. In circumstances where HIV-positive mothers choose not to breastfeed, the provision of acceptable, feasible, affordable, sustainable and safe replacement foods is recommended (WHO, 2003b).

2.2 Complementary feeding of infants and young children in developing countries

2.2.1 Complementary feeding practices

Inappropriate feeding practices have been identified among the causes of malnutrition in children in developing countries (WHO, 2003a). In many developing countries, complementary foods are introduced too early or too late and the quality and quantity of the foods are insufficient leading to a great risk of nutritional deficiencies during the second half of infancy (Pelto et al, 2003). A recent survey of complementary feeding practices in Central Durban, South Africa (Kassier et al, 2003) showed that exclusive breastfeeding was the predominant method of child feeding for the first 6 weeks of life after which mixed feeding was predominantly practiced through to 6 months. A longitudinal study from Peru showed that only 12% of infants were exclusively breastfed at 1 month of age (Brown et al, 1989). The study further showed that the introduction of fluids other than breast milk before the age of 6 months doubled the prevalence of diarrhea. A prospective observational study from Bangladesh (Arifeen et al, 2001) demonstrated that exclusive breastfeeding for the first few months of life reduced

the risk of infant mortality associated with acute respiratory infection (ARI), diarrhea and all causes by 2.4- , 3.9- and 2.2-fold, respectively.

2.2.2 Complementary foods

The World Health Organisation (WHO, 1996) defines a complementary food as any food, whether manufactured or locally prepared, suitable as a complement to breast milk or to infant formula, when either becomes insufficient to satisfy the nutritional requirements of the infant. Such food is also commonly called ''weaning food'' or ''breast-milk supplement''.

Provision of safe and appropriate complementary foods processed from indigenous nutrient-rich foodstuffs is currently emphasized (WHO, 2002). The most common forms of processed complementary foods are semi-solid gruels or porridges. These are generally prepared in two steps. The first step consists of processing the available staple food (cereal, root or tuber) and major protein source (usually le.g.ume or oil seed) into intermediate flours or doughs, which can be stored for variable lengths of time. During this first step several unit operation procedures are undertaken including cleaning and physical separation of inedible or undesirable parts of the raw foods, size reduction, drying, and possibly pre-cooking and enzymatic treatment. The second step consists of the actual preparation and cooking of the mixtures from the intermediate products and generally takes place at home, although it can also be done at local health or community centre (WHO, 1998).

2.2.2.1 Home-prepared (traditional) complementary foods

It is difficult to achieve a safe and nutritionally adequate diet for 6 - 24 - month old children (Mensah and Tomkins, 2003) due to the fact that most of the traditionally used complementary foods in developing countries are unfortified (Hotz and Gibson, 2001) cereal-based gruels characterised by high water content, and low energy and nutrient density (Gibson et al, 1998, den Besten et al, 1998). The high volume/high-viscosity characteristic of cereal-based foods is commonly referred to as dietary bulk (Hansen et al. 1989). Most of the gruels prepared in households have an energy density of about 40 kcal/100 g (Vieu et al, 2001; Chakravarthi and Kapoor, 2003) and may have to be fed frequently due to the limited gastric capacity of infants (Vieu et al, 2001). For example, the dry matter content of *nshima* (stiff porridge prepared from maize meal), the main staple in Zambia, is 0.39 (SD 0.03) g/g (Hayes et al, 1994). This corresponds to an energy density of 47.9 kcal/100 g of *nshima*. Hayes and co-workers (Hayes et al, 1994) also reported that the energy densities of Zambian porridges prepared from maize meal alone range from 14.4 – 31.7 kcal/100 ml, 26.7 – 58.8 kcal/100 ml and 35.2 - 73.5 kcal/100 ml for porridges of thin, medium and thick consistency.

One way of reducing viscosity and ensuring higher nutrient density in complementary foods is through the addition of α-amylase. A wide range of suitably stable, food-grade amylases are commercially available at reasonably low cost and can be added to dried flours during blending at the cottage industry or larger industrial scale level or at the time of cooking in the household (Brown et al, 1998).

2.2.2.2 Naturally occurring toxins and nutrient inhibitors in complementary foods

Most complementary foods in developing countries are based on cereals, le.g.umes and starchy roots and tubers. Both cereals and le.g.umes contain inherent anti-nutrients such as phytic acid (Hurell et al, 2000; Onyeike and Omubo-Dede, 2002), oxalates, poly-phenolic compounds such as tannins, and trypsin inhibitors (Massey et al, 2001; Onyeike and Omubo-Dede, 2002). These anti-physiological factors adversely affect the availability of nutrients from foods. For example, phytate strongly inhibits the absorption of iron (Hurell et al, 2000) and zinc (IZiNCG, 2004), while polyphenols are known to inhibit iron absorption (Hurell et al, 1999) and may also have adverse effects on food color and flavor (Salunkhe et al, 1982). While domestic processing techniques such as cooking, soaking, dehulling and autoclaving result in significant reductions in polyphenolic compounds, trypsin inhibitor and oxalate (Onyeike and Omubo-Dede, 2002; Obizoba and E.g.buna, 1992; Sharma and Sehgal. 1992; Salunkhe et al, 1982), it is apparent that these procedures have lesser effect on phytate (Trugo et al, 2000).

2.2.2.2.1 Aflatoxins

In addition to anti-physiological factors, staple foods in developing countries are also most often contaminated with aflatoxins, secondary fungal metabolites that are known to be both toxic and carcinogenic (Turner et al, 2005). Aflatoxins are mycotoxins produced by certain fungi, especially, *Aspergillus flavus* and *Aspergillus parasiticus* (Akiyama et al, 2001), which grow on food and feed crops. Aflatoxins have closely related structures and are highly oxygenated, naturally occurring heterocyclic compounds (Heathcote, 1978). There are four naturally occurring aflatoxins (Figure 2-1) namely, aflatoxin B_1, B_2, G_1 and G_2

(Garrido et al, 2003). When ruminants eat foodstuffs containing aflatoxins B_1, this toxin is metabolized and excreted after hydroxylation as, a comparatively less toxic, aflatoxin M_1 in milk (Garner et al, 1993).

Although aflatoxins were first discovered as contaminants of groundnuts and groundnut products, it is now known that maize is the most important source of aflatoxin exposure (Rodricks and Pohland, 1981) due the wide consumption of maize and maize products. Low levels of aflatoxins have been reported in soybeans (Sobolev and Dorner, 2002) and aflatoxin residues have been found in fluid milk and non-fat dry milk (Bullerman, 1979). The growth and aflatoxin production by aflatoxigenic moulds depend on several factors including the storage temperature, moisture content and water activity of the product (Garner et al, 1993; Moss, 1991). The minimum, optimum and maximum growth temperature ranges for aflatoxigenic moulds are 6 – 8 °C, 36 – 38 °C and 44 – 46 °C, respectively (Garner et al, 1993).

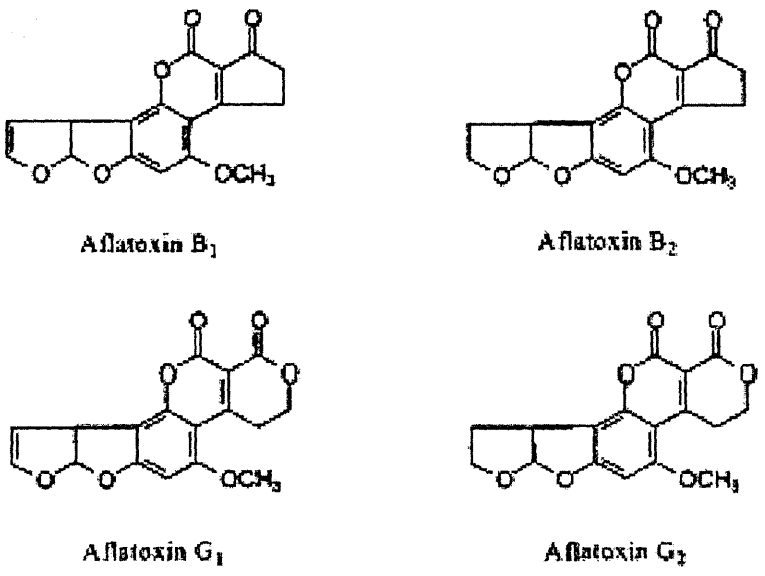


Figure 2-1 Chemical structures of aflatoxins B_1, B_2, G_1 and G_2

2.2.2.2.1.1 Effects of dietary aflatoxin exposure in infants and young children

Aflatoxins are known for their acute toxicity and carcinogenicity (Jaimez et al, 2000; Hall and Wild, 2003; Nasir and Jolley, 2000). They are associated with immune function modification (Turner et al, 2003), stunting (Gong et al, 2002) and disorders in protein and enzyme synthesis and lipid metabolism and kwashiokor (Hendrickse, 1984). Maize and groundnuts, the main ingredients used for complementary foods in most African countries, are most vulnerable to aflatoxin contamination (Gong et al, 2003). Aflatoxin exposure in children is strongly associated with the introduction of non-breast milk foods (Gong et al 2003). This is of relevance for infants of HIV-infected mothers who are likely to be fed non-breast milk foods early due to knowledge of risk of HIV transmission through breast milk (Omari et al, 2003; Chisenga et al, 2005). Aflatoxin exposure in infants may exacerbate the adverse effects of HIV exposure such as growth faltering (Apardi, 2000) and immunological deficiencies (Nielsen et al, 2001). While groundnuts are often contaminated, sorting (physical removal of mouldy kernels) may be used to reduce aflatoxin levels. Heat extrusion may also reduce aflatoxin levels (Castells et al, 2005).

2.2.2.2.2 Phytic acid

Phytic acid (Myo-inositol hexaphosphate) is composed of six phosphate esters (IZiNCG, 2004). Phytate, the magnesium, calcium or potassium salt of phytic acid IZiNCG, 2004) is the primary storage form of phosphorus and occurs in high concentrations in cereal grains, nuts and le.g.umes (Hidve.g.i and Lasztity, 2002, IZiNCG, 2004) with lower concentrations in fruits and ve.g.etables (IZiNCG, 2004). The highest phytic acid concentration in most grains is found in the bran (Hidvegi and Lasztity, 2002; Hurrell, 2003). Due to the fact that phytate cannot be

digested or absorbed in the human intestinal tract, minerals bound to phytate also pass through unabsorbed (IZiNCG, 2004b). It is important to reduce phytate concentration in foods or inhibit the formation of phytate-mineral complexes.

The effects of phytic acid on nutrient absorption can be reduced in several ways including enzymatic degradation (Hurrell et al, 2003; Sandberg, 2002; Hurrell, 2003), adding to food compounds such as ascorbic acid or sodium EDTA that prevent phytate-mineral complex formation and milling of cereals to remove the bran fraction (Hurrell, 2003). Since phytate binds minerals, especially zinc and iron in a dose-dependant manner, it is possible to calculate phytate-to- mineral molar ratios (IZiNCG, 2004b) that can be used to gauge the relative bioavailability of such micronutrients from a food. For example zinc absorption is classified to be high, moderate or low when the phytate:zinc molar ratio is < 5, 5-15, or > 15, respectively. Typical phytate:zinc molar ratios range from 22-88 for seeds, le.g.umes, nuts and unrefined cereal grains and 0-42 for other plant foods (IZiNCG, 2004b).

2.2.2.3 Centrally-processed multi-micronutrient fortified cereal-based complementary foods

The World Food Programme (WFP) distributes processed complementary foods or blended foods including Unimix, Indiamix and Lakuni Phala for use in maternal and child health programs targeting poor families in developing countries (Dijkhuizen, 2000; Ruel et al, 2004). WFP provides product specifications and processing instructions to local manufacturers who produce these blends from locally available ingredients, with the exception of multi-

micronutrient premixes which are purchased from international suppliers (Dijkhuizen, 2000).

The quality of cereal-based complementary foods may be improved through central processing in order to ensure hygiene (Dewey and Brown, 2003). Secondly, techniques such as modification of complementary foods with α-amylase to enhance food intake by enabling the addition of more flour per unit volume of water and multi-micronutrient fortification to enhance nutrient density are more feasible if complementary foods are centrally processed. Heat stable food-grade α-amylase can be obtained easily in developing countries at approximately US$75/ kg. 1 kg of α-amylase may be used to process approximately 2000 kg of complementary food. Thermal processing techniques such as extrusion cooking may not only reduce cooking time during food preparation but may also result in lower microbiological count, lower levels of anti-nutrients and aflatoxins in complementary foods.

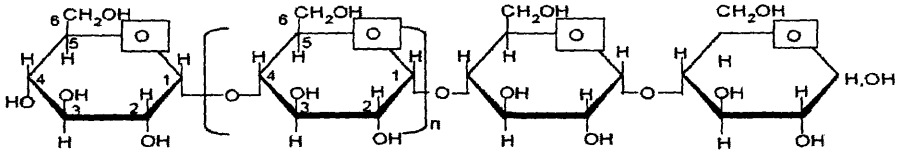
Although such complementary food processing processes are unlikely to benefit rural poor communities, they will be appropriate for more households due to the global increase in urbanization.

2.2.2.3.1 Starch chemistry and the action of α-amylase

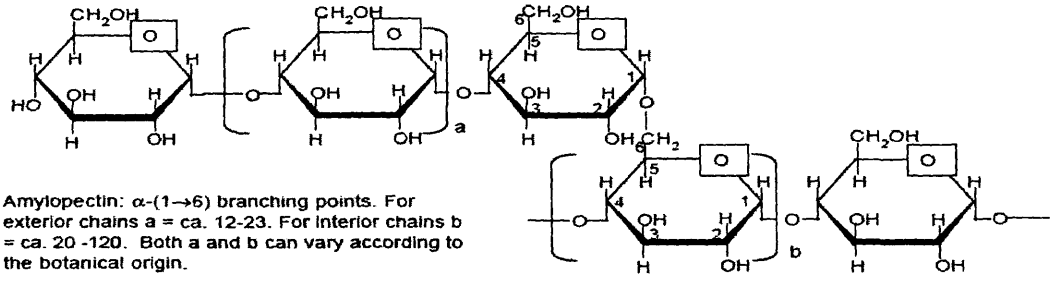
2.2.2.3.1.1 Starch chemistry

Pure starch consists predominantly of two molecules namely, amylose and amylopectin (Bentley, 1999; Hoover, 2001; Tester et al, 2004) (Figure 2-2). Amylose is a roughly linear molecule containing ~99% α-(1-4) and ~1% α-(1-6) bonds with up to 1000 glucose units. Amylopectin is a much larger molecule than

amylose and is heavily branched with ~95% α-(1-4) and ~5% α-(1-6) and about 12 – 120 glucose units. Starches are defined as waxy when the ratio of amylose to amylopectin is low (~15%), normal when amylose represents ~16-35% and high-amylose (or amylo-) when amylose exceeds ~36% (Tester et al, 2004).



Amylose: α-(1→4)-glucan; average n = ca. 1000. The linear molecule may carry a few occasional moderately long chains linked α-(1→6).



Amylopectin: α-(1→6) branching points. For exterior chains a = ca. 12-23. For interior chains b = ca. 20 -120. Both a and b can vary according to the botanical origin.

Figure 2-2 Starch chemistry (taken from Tester et al, 2004)

In starch granules, amylose and amylopectin are linked together through hydrogen bonding, which gives native starch its insolubility in water (Bentley, 1999).When starch is heated in the presence of excess water, undergoes an order–disorder phase transition called gelatinization (Hoover, 2001; Tester et al, 2004). Gelatinization typically begins at ~45°C (onset temperature), peaks at 60°C and is complete at 75°C (Tester et al, 2004). This process makes the starch completely digestible by starch hydrolysing enzymes such as α-amylase over a temperature range characteristic of the starch source (Hoover, 2001).

2.2.2.3.1.2 α-amylase

α-amylases (endo-1,4-α-D-glucan glucohydrolase) are extracellular enzymes that randomly cleave the 1,4-α-D-glucosodic linkages (Figure 2-2) between adjacent glucose units in the linear amylose chain (Pandey et al., 2000). The end products of α-amylase action are oligosaccharides and α-limit dextrins (Van der Maarel et al., 2002). α-amylase can be used in complementary foods in two forms, namely, amylase rich flour (ARF) and as commercial preparation (industrial food-grade amylase).

2.2.2.3.1.3 Amylase Rich Flours

Amylase rich flours are made by germinating (malting) of cereal grains. During germination, α-amylase activity is developed. A small amount of flour made of germinated grain is then added to non-germinated cereal flour in order to achieve viscosity reduction (Hansen et al, 1989). The benefits of amylase-rich flour (ARF) in improvement of energy density and reduction of viscosity of complementary blends have been reported (Tontisirin and Yamborisut, 1995; den Besten et al., 1998). Higher intake of the ARF-treated gruels has been associated with substantial improvement in child growth (Tontisirin and Yamborisut, 1995, Gopaldas and John, 1991; John and Gopaldas, 1993). The promotion of ARF for use in complementary foods in developing countries has been limited by several factors namely, 1) long preparation duration, 2) cultural obstacles to the use of ARF due its use in alcohol processing, and 3) high risk of toxicity from hydrocyanide (in sorghum) and aflatoxins (Ashworth and Draper, 1992; Brown et al 1998). It takes upto 72 hours to prepare ARF (Ashworth and Draper, 1992) hence the process may be associated with high opportunity costs. The risk of cyanide production is restricted to sorghum that is rich in dhurrin, yielding

hydrocyanic acid on hydrolysis. Exposure to hydrocyanide is associated with goiter and high doses are fatal (Ashworth and Draper, 1992). The use of commercially available α-amylase preparations may be a suitable alternative to ARF.

2.2.2.3.1.4 Industrial food-grade α-amylase

A wide range of suitably stable, food-grade amylases are commercially available at reasonably low cost and can be added to dried flours during blending at the cottage industry or larger industrial scale level or at the time of cooking in the household (Brown et al., 1998). α-amylase is found in a wide variety of microorganisms, and may be derived from several bacteria, yeasts and fungi. Strains of *Aspergillus sp.* and *Bacillus sp.*, mainly *Bacillus amyloliquefaciens* and *Bacillus licheniformis*, are employed for commercial applications (Pandey et al., 2000). Bacterial α-amylases produced from *Bacillus subtilis* or *Bacillus amyloliquefaciens* have an optimum operating temperature between 70 and 75 °C and may be used up to a maximum of 90 °C. However, bacterial α-amylases produced by *Bacillus licheniformis* or *Bacillus stearothermophilus* may be used up to 110 °C (Bentley, 1999).

2.2.2.3.1.5 Effect of α-amylase treatment on children's nutrient intake and nutritional status.

Table 2-2 gives a summary of some studies assessing the effect of amylase on children's nutrient intake and nutritional status. The studies were identified based on systematic literature search on Pubmed by entering different combinations of key words or phrases, namely, amylase, amylase rich flour, infant, children,

growth, nutrient intake, energy density and viscosity. All studies describing amylase as an enzyme and review papers were excluded from the analysis.

The studies concentrated mainly on the use of amylase rich flours (ARF) and were mainly carried out among disadvantaged populations. Secondly most of the studies were designed to provide short-term intervention. The studies show that in circumstances where infants and children are severely malnourished, the addition of amylase to complementary foods or the use of amylase rich flour may result in improved energy intake and growth. There is need for further investigation of the benefits of α-amylase in increasing nutrient intake by infants.

Table 2-2 Effect of α-amylase treatment of complementary foods on children's nutrient intake and growth.

Reference	Country	Age (months)	Duration	N	Design	Setting	Results
den Besten et al, 1998	South Africa	10-24		30	Self-controlled clinical trial of effect on dietary intake of addition of α-amylase and extra cereal to a diet including three meals.	Healthy children	Amylase treatment resulted in 24% and 10% increase in energy and protein intake, respectively
Moursi et al, 2003	The Congo	4	4.5 months	75	Randomized controlled trial (α-amylase-treated maize-soya blend or similar flour without α-amylase)	Urban borough of Brazzaville, premature introduction of complementary foods, low daily feeding frequency, high stunting prevalence (15.5%)	Significantly greater energy intake from amylase-treated gruel at 24 weeks. No difference in total energy intake Significantly greater length gain in amylase-treated group between 24-31 weeks of age No differences in weight gain
Mensah et al 1995	Ghana and Nigeria	6-15		40	Randomized clinical using three porridges namely, 1) fermented maize-soybean with amylase 2) non-fermented maize-soybean with amylase and 3) fermented maize	Infants admitted with acute diarrhea in peri-urban Ghana and urban Nigeria.	Higher acceptance of fermented maize porridge No difference in children's porridge intake Higher daily nutrient intake in intervention groups due to doubled solids content

Table 2-2 cont.

Reference	Country	Age (months)	Duration	N	Design	Setting	Results
Rahman et al, 1997	Bangladesh	6-11	72 hours	28	Randomized controlled trial on the effect on nutrient absorption. (energy dense diet versus porridge diluted with water)	Children with acute watery diarrhea	Higher protein, fat and energy intake, higher protein absorption coefficient and positive nitrogen balance in energy dense group
Darling et al 1995	Tanzania	6-25 months	4 days	75	Controlled clinical trial (amylase-digested, fermented-amylase-digested or conventional maize porridges	Children admitted to hospital with acute diarrhea given diets ad libitum five times daily	42% higher energy intake in amylase-digested than conventional group ($p = 0.003$) No differences in duration of diarrhoea, frequency of stooling or vomiting
Rahman et al, 1994	Bangladesh	5-18	5 days	78	Randomized controlled trial (ARF-digested energy-dense porridge, an unaltered thick porridge of similar energy density, or water-diluted porridge with same viscosity as first diet)	Severely malnourished children in a nutrition rehabilitation unit of a large diarrhoea treatment centre. Children also received an additional three milk-cereal meals a day.	Significantly higher daily energy intake in ARF-digested group than both control groups.

Table 2-2 cont.

Reference	Country	Age (months)	Duration	N	Design	Setting	Results
Gopaldas and John, 1991	India	6-24	6 months	68	Randomized controlled trial on the effect on growth. (ARF-digested wheat flour (1.63 kcal/ml) versus identical high viscosity gruel)	Slum-dwelling children given once-a-day ad lib feed in addition to traditional home diet	Higher porridge and energy intake (124 ml and 199 kcal) than control group (31 ml and 50 kcal), respectively.
John and Gopaldas, 1993	India	6-24	6 months	68	Randomized controlled trial on the effect on nutrient intake. (ARF-digested wheat flour (1.63 kcal/ml) versus identical high viscosity gruel)	Slum-dwelling children given once-a-day ad lib feed in addition to traditional home diet	Significantly greater weight and height in experimental group.

2.2.2.3.2 Application of extrusion cooking in complementary food processing

Another technique that can be used to reduce viscosity and enhance nutrient density in complementary foods is extrusion cooking. Extrusion cooking has been used in developing countries since 1950s to process cereal-soy blends such as Pronutro (South Africa), Faffa (Ethiopia), Superamine (Algeria and E.g.ypt), Multipurpose Food (India), Nutripak (Philipines) and Speciality Baby Porridge (Zambia) (Royal Tropical Institute, 1983).

During extrusion the uncooked food material is fed into the extruder (Single screw X20, 3.25 inch barell, 220-1000 kg/h, Wenger Manufacturing Inc, Sabetha, USA) in which it is subjected to intense mechanical shear, worked into viscous, plastic like dough and cooked before being forced through a die (Alonso et al., 2000a; Cabrejas-Martin et al., 1999) at high temperatures (150 - 180 °C) and pressure (up to 25 mPa) for 60 – 120 seconds at a moisture content of about 20% (WHO, 1998).

Beneficial effects of extrusion cooking include improvement in protein digestibility and the bioavailability of sulphur-amino acids (WHO, 1998), reductions in microbial count, improved palatability and texture (Martin-Cabrejas et al., 1999) and enhanced starch digestibility (Alonso, et al., 2000a, Malleshi et al., 1996, Omueti and Morton, 1996). Extrusion cooking has been suggested as one way of reducing dietary bulk in weaning foods (Treche and Mbome, 1999; Iwe, 1998) possibly due to thermal hydrolysis of starch (Moraru and Kokini,

2003). It might also be used to eliminate anti-nutrients that occur in foods. (Cazzaniga et al. 2001).

Several studies have reported elimination or reduction in concentrations by extrusion cooking of lectins such as haemaglutinins (Alonso et al 2000a; Martin-Cabrejas et al 1999), trypsin inhibitor (Marzo et al., 2002; Alonso et al 2000a), α-amylase inhibitor (Martin-Cabrejas et al 1999; Marzo et al., 2002; Alonso et al 2000b), hemaglutinnins (Marzo et al., 2002; Alonso et al 2000b), and condensed tannins (Marzo et al., 2002; Alonso et al 2000b). Some studies have reported (Chauhan et al. 1988, Marzo et al 2002) reduction in phytate content of cereals and le.g.umes processed by extrusion cooking. A few studies (Buser and Abbas, 2002, Elias-Orozco et al, 1988) have reported reduction in aflatoxin content following food extrusion.

Extrusion has been reported to result in decreased apparent absorption of Zn, Mg and P (Kivitso et al., 1986) and in insignificant decrease in iron absorption (Hurrell et al., 2002). This may be due to the fact that extrusion cooking results in deactivation of phytase resulting in only about 25% digestion of phytate or due to the formation of indigestible phytate complexes (Sandberg et al, 1987). This apparent decrease in micronutrient absorption by extrusion cooking and thermal treatment of food may be mitigated by the multi-micronutrient fortification of complementary foods.

2.2.2.3.3 Multi-micronutrient fortification of complementary foods

Although pulses contain iron, its availability is low and it is now known that it is difficult to meet infants'needs for micronutrients such as iron, zinc and calcium

without the provision of fortified foods (WHO, 2004). The Food and Agricultural Organization (FAO, 1996) Codex Alimentarius defines food fortification as "the addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups".

Food fortification can be mandatory by le.g.islation or it can be voluntary, that is, left to the discretion of the food manufacturer. More than 25 countries worldwide currently require mandatory fortification of one or more foods with micronutrients, including vitamin A and iron, and several other countries practice voluntary food fortification (ILSI, 2003). Table 2-3 shows food fortification programs in some African countries up to 2004. The rate of adoption of food fortification is not uniform in all developing countries due to lack of political will in some countries and variation among countries in the recognition of the importance of micronutrients for child survival and later work capacity. It has been noted that nutrition is rarely high on the political agenda of most governments in developing countries (WFP, 2006a).

Table 2-3 Food fortification programs in some African countries up to 2004*

Country	Program**	Vitamins and minerals added
Burkina Faso	Cotton seed oil[1]	vitamin A
Cote d'Ivoire	Wheat[1]	Folic acid, iron
	Edible oils[1]	vitamin A
Ghana	Wheat[2]	vitamins A, B1, B2, B6, B12, folic acid, niacin; iron, zinc
	Edible oils[2]	vitamin A
Guinea	Wheat[2]	vitamins B1, B2, folic acid, niacin; iron
Kenya	Maize[1]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
	Edible oils[1]	vitamin A
Lesotho	Wheat[2]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
	Maize[2]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
Malawi	Maize[2]	vitamins A, B1, B2, B6, B12, folic acid, niacin; iron, zinc
	Sugar[2]	vitamin A
Mali	Cotton seed oil[2]	vitamin A
Morocco	Wheat[3]	vitamins B1, B2, folic acid, niacin; iron
	Edible oils[2]	vitamins A, D
Namibia	Maize[2]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
Nigeria	Wheat[3]	vitamins A, B1, B2,niacin; iron
	Maize[3]	vitamin A
	Edible oils[3]	vitamin A
	Sugar[3]	vitamin A
South Africa	Wheat[3]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
	Maize[3]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
Sudan	Wheat[2]	Folic acid, iron
Uganda	Wheat[1]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
	Maize[2]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
	Edible oils[1]	vitamin A
	Sugar[1]	vitamin A
Zambia	Wheat[2]	vitamins B1, B2, niacin
	Maize[2]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
	Sugar[3]	vitamin A

* taken from van Ameringen, 2005. ** 1 = pending; 2 = voluntary; 3 = mandatory

Table 2-3 Food fortification programs in some African countries up to 2004*

Country	Program**	Vitamins and minerals added
Burkina Faso	Cotton seed oil[1]	vitamin A
Cote d'Ivoire	Wheat[1]	Folic acid, iron
	Edible oils[1]	vitamin A
Ghana	Wheat[2]	vitamins A, B1, B2, B6, B12, folic acid, niacin; iron, zinc
	Edible oils[2]	vitamin A
Guinea	Wheat[2]	vitamins B1, B2, folic acid, niacin; iron
Kenya	Maize[1]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
	Edible oils[1]	vitamin A
Lesotho	Wheat[2]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
	Maize[2]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
Malawi	Maize[2]	vitamins A, B1, B2, B6, B12, folic acid, niacin; iron, zinc
	Sugar[2]	vitamin A
Mali	Cotton seed oil[2]	vitamin A
Morocco	Wheat[3]	vitamins B1, B2, folic acid, niacin; iron
	Edible oils[2]	vitamins A, D
Namibia	Maize[2]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
Nigeria	Wheat[3]	vitamins A, B1, B2,niacin; iron
	Maize[3]	vitamin A
	Edible oils[3]	vitamin A
	Sugar[3]	vitamin A
South Africa	Wheat[3]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
	Maize[3]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
Sudan	Wheat[2]	Folic acid, iron
Uganda	Wheat[1]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
	Maize[2]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
	Edible oils[1]	vitamin A
	Sugar[1]	vitamin A
Zambia	Wheat[2]	vitamins B1, B2, niacin
	Maize[2]	vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc
	Sugar[3]	vitamin A

* taken from van Ameringen, 2005. ** 1 = pending; 2 = voluntary; 3 = mandatory

Food fortification has been used to control micronutrient deficiencies in the industrialised world (Darton-Hill, 1998; Chen and Oldewage-Theron, 2002). An example is the fortification of flour and bread with niacin in the United States that is believed to have been instrumental in reducing pellagra-attributed mortality in the 1930s and 1940s and then eliminating pellagra. In the United Kingdom, the Department of Health recently recommended that flour be fortified with folic acid at 2,400 μg/kg, which is projected to increase folic acid intake by 191μg/day in adolescent girls (ILSI, 2003).

In Guatemala, about 55% of the total vitamin A intake of toddlers from non-breast milk food sources is derived from mandatory fortification of three foods namely, sugar, Incaparina (a blended maize-based product), and margarine (ILSI, 2003). Margarine had been fortified in Zambia since 1978, but this program had not contributed greatly to the vitamin A levels in the population due to low margarine consumption levels, especially among the poor (Serlemitsos and Fusco, 2001).

The effectiveness of a food-fortification programme depends on whether or not the fortified food is accepted, purchased, and consumed by the targeted population. The quality, taste, and the price of the fortified product play important roles in determining the effectiveness of the fortification programme (Wirakartakusumah and Hariyadi, 1998). Several factors that should be considered carefully in designing food fortification programmes (Wirakartakusumah and Hariyadi, 1998) include, 1) the food chosen as the carrier should be consumed in sufficient quantities to make a significant contribution in the diet of the target population; 2) the addition of nutrients should not create an

imbalance of essential nutrients, especially with reference to multiply fortified foods in which interaction among the added nutrients (and also among the added and the nutrients that are naturally present in the food carrier) is likely to occur; 3) the added nutrient should be stable under normal conditions of storage and use; 4) the price of the fortified food should be affordable for the targeted population; 5) programmes of quality assurance and control of fortified food can be more easily implemented if the fortification programme is centralised and involves mass production; 6) the food should be distributed to as much of the target population as possible.

2.2.2.3.3.1 Effect of complementary food fortification on infant health and nutrition

There are currently very few studies on the nutritional and health effects of fortified complementary foods, especially in children living in middle income urban settings in developing countries. Table 2-4 summarizes some studies that have assessed the effect of fortified complementary foods on growth and micronutrient status of infants and young children. The need to conduct well-designed studies to demonstrate the effectiveness of fortification has been highlighted (Townbridge and Martorell, 2002).

Recent work from Haiti (Ruel et al, 2004) assessed the benefits of fortified cereal blends such as corn-soy blend (CSB) or wheat-soy blend (WSB) in improving the quality of the diet of infants and young children 6 to 23 months of age. Participatory recipe trials were conducted to assess current complementary feeding practices in the Central Plateau of Haiti and to develop new, improved recipes by using a combination of locally available ingredients and foods and donated fortified cereal blends. The results showed that only preparations using

CSB could achieve the recommended concentrations of iron and zinc in complementary foods for young children 12- to 23 months old. In contrast, the iron and zinc needs of infants, especially those between 6 and 8 months of age, could not be met, even with a combination of fortified CSB and other locally available, acceptable, and affordable foods. The workers recommended higher fortification levels if iron and zinc needs of infants are to be met. This study did not include determination of micronutrient status.

A study from Brazil (de Almeida et al., 2003) evaluated the efficacy of fortified orange juice in 50 preschool children. The children received two flasks of 200ml orange juice fortified with 20mg ferrous sulphate hepta-hydrate, providing 2mg elemental iron/100ml for a total of 84 days. Capillary haemoglobin concentration, weight-for-age, weight-for-height and height-for-age were determined at the be.g.inning of the study and after 4 months. There was an increase in haemoglobin concentration from 10.4 to 11.6 g/dL and a decrease in the prevalence of anaemia from 60 to 20%. There were no significant differences in growth Z-scores.

Table 2-4 Effects of multi-micronutrient fortified complementary foods on growth and micronutient status of infants and young children

Reference	Country	Age (months)	Duration	N	Design	Setting	Results
Lartey et al, 1999	Ghana	6	6 months	208	Randomized community-based trial (4 centrally processed complementary foods namely, 1) weanimix with vitamins and minerals, 2) Weanimix only, 3) Weanimix with fish powder, and 4) Koko with fish powder; Cross sectional data before and after on 464 infants not included in intervention	Rural breastfed infants	No differences among intervention groups in growth and micronutrient status. Significantly higher weight-for-age and length-for-age in combined intervention from 9 -12 months. Significantly greater change in plasma retinol in fortified group and lower proportion of infants with low ferittin values in Weanimix than other diets
Torrejon et al, 2004	Chile	12	at least 6 months	42	Self-controlled trial (cow's milk fortified with iron (10 mg/L, zinc (5 mg/L and copper (0.5 mg/L))	Healthy male children with normal growth and from lower socioeconomic groups	Favourable effect on iron status, but no effect on zinc status
Oelofse et al, 2003	South Africa	6	6 months	60	Randomized controlled trial (fortified complementary food versus controls on traditional diet.	Urban disadvantaged black community.	Significantly higher serum retinol at 12 months. Less pronounced decline in serum iron. No effects on haemoglobin concentration, weight or length gain.

Table 2-4 cont.

Reference	Country	Age (months)	Duration	N	Design	Setting	Results
Nesamvuni et al, 2005	South Africa	12-36	12 months	44	Randomized parallel, single-blind intervention (Maize meal fortified with vitamin A, thiamine, riboflavin and pyridoxine versus unfortified maize meal)	Children with height-for-age or weight-for-age below the 5th percentile of the NCHS criteria in a small town and attending local crèche or clinic	Significant weight gain in experimental group. No significant differences in haemoglobin concentration and serum retinol. Significant decrease in retinol binding protein in control group.

In a randomized, double-blind, placebo-controlled efficacy trial (Ash et al., 2003) of a beverage fortified with 10 micronutrients in Tanzanian, children 6-11 years old, were assigned to receive the fortified or unfortified beverage at school for 6 mo. At 6 months follow-up among children with anemia (haemoglobin concentration < 110 g/L), there was a significantly larger increase in haemoglobin concentration in the fortified group than in the unfortified group (9.2 and 0.2g/L, respectively). There was a cure rate for anemia of 21%. The prevalence of children with low serum retinol concentrations (< 200μg/L) decreased from 21.4% to 11.3% in the fortified group compared with a non-significant change (20.6% to 19.7%) in the non-fortified arm. There were also significantly higher increments in weight, height and BMI. In Botswana (Abrams et al., 2003), a total of 311 children 6-11 years old were given seven 240-ml servings weekly of either a beverage fortified with 12 micronutrients or an iso-energetic placebo drink for 8 weeks. Weight, mid-upper arm circumference, haemoglobin concentration, retinol, ferritin, vitamin B-12, folate and riboflavin status were measured both at baseline and at the end of the study. There were significantly higher changes in anthropometric indicators in the fortified beverage arm. Ferritin, riboflavin and folate status were also significantly better in the fortified beverage group, but thiamine status was not. Zinc was significantly higher, while transferrin receptors were significantly lower at the end of the study in the fortified beverage group. There was no change in plasma retinol concentrations.

The studies above show that fortification improves haematological measurements in pre-school and primary school children. However, the results on growth and serum retinol are inconsistent. These differences can be explained by the

coexistence of micronutrient deficiencies and micronutrient interactions. The amounts of micronutrients in a fortified complementary food also affect the outcomes.

2.3 Measurement of nutrient intake of infants and young children

The difficulty in accurately measuring food intake of infants has been highlighted (Dewey and Brown, 2003). Knowledge of milk intake by infants is of basic biological interest and is required for the estimation of daily nutrient intake during this period of an infant's life (Hendrickse and Wamberg, 1999). The dearth of published breast milk intake data for older infants in low-income countries has been underscored (Dewey and Brown, 2003).

Complementary foods have been shown to partially displace breast milk (Bajaj et al, 2005) and may also interfere with absorption of nutrients from breast milk (Brown et al, 1995). This may result in greater likelihood of nutrient deficiencies if the density and bioavailability of nutrients in the complementary foods are not equal to or greater than those of breast milk (Brown et al, 1995). However, multi-micronutrient fortified, nutrient-dense complementary foods may partially displace less nutritious traditional foods resulting in increased energy and nutrient intake.

In studies of food intake of breast-fed infants, both breast milk intake and intake of complementary foods need to be accurately assessed (Haisma et al, 2003). Such data can be used in designing the nutrient composition of a fortified processed

food. Secondly, availability of data on the amount of complementary food consumed in grams and kilocalories (Lutter, 2003) will be the basis for the recommendation of daily rations. Such information can also be used to educate mothers and caretakers on sound weaning practices that can help prevent early growth faltering.

2.3.1 Complementary foods intake measurement techniques

Dietary intake assessment techniques include self-reported data from questionnaires and interviews (Johnson 2002, Gibson and Ferguson, 1999, Subar et al, 2003), biological markers such as urinary nitrogen and potassium, circulating nutrients in blood such as ascorbic acid and doubly labeled water for total energy expenditure (McKeown et al, 2001; Subar et al, 2003). Since biomarkers are influenced by intervening factors such as smoking status and use of supplements, they do not reflect absolute dietary intakes and can only be used to validate other dietary assessment methods (McKeown et al, 2001).

Dietary assessment methods based on self-reported data include observer-recorded food records, food frequency questionnaires and 24-hour recalls (Johnson, 2002; Hise et al, 2002). Although techniques based on self-reported data have been associated with underestimation of nutrient intake (Trabulsi and Schoeller, 2001; Haisma et al, 2005), they are used widely due to the fact they are relatively inexpensive and are easy to administer (Subar et al, 2003; McKeown et al, 2001). Food records completed by trained research assistants have been the most commonly used method to quantify dietary intake in developing countries (Gibson and Ferguson, 1999). However, food records are expensive, time consuming and invasive and might result in decreased response rates, response

bias and loss of statistical power and a possibility of change in dietary intake during the record period (McNaughton et al, 2005; Gibson and Ferguson, 1999; Gibson and Ferguson, 1998).

Food frequency questionnaires (FFQs) are used to estimate dietary intakes for groups over time and to place individuals into broad cate.g.ories based on a distribution of nutrient intake (Johnson, 2002). The advantages of FFQs include relatively lower administrative costs and time and the ability to assess usual and longer term intake. The disadvantages include increased literacy demands and respondent burden, inaccuracy of absolute nutrient values, fluctuation of nutrient values depending on instrument length and structure and lack of detail re.g.arding specific foods (Resnicow et al. 2000).

24-hour recall is used to quantitatively assess current nutrient intake and is brief lasting 20 to 30 minutes with less respondent burden (Johnson, 2002; Gibson and Ferguson, 1999). While repeated 24-hour recalls may be used to assess actual food intakes for both individuals and groups, single 24-h recalls are applicable for the determination of group food intakes. 24-recall may also be used to measure dietary enhancers or inhibitors that influence nutrient absorption (Gibson and Ferguson, 1999). This technique is appropriate in low-resource settings because subject literacy is not necessary (Johnson, 2002). The feasibility and validity of the 24-hour recall method have been assessed (Gibson and Fergusson).

2.3.2 Measurement of breast milk intake

Breast milk intake has been traditionally measured by test weighing that involves weighing the infant or the mother before and after breastfeeding (Arthur et al, 1987; Coward et al, 1979; Coward, 1984). Test-weighing is inaccurate especially in developing countries where breastfeeding frequency is high and feed volumes low (Coward, 1984). Further, this technique may interrupt customary feeding patterns and normal mother-infant interaction (Coward et al, 1979).

Through developments in stable isotope technologies it is now possible to measure the dietary energy available to the infant under field conditions. It is feasible to use the deuterium dose-to-the-mother method for estimation of breast milk transfer to the infant (Coward et al, 1982). Deuterium dilution is a non-invasive, simple, safe and accurate method for measuring breast milk transfer. It is applicable, especially for estimating breast milk contribution to nutrient intake in non-exclusively breastfed infants (Cisse et al, 2002). It also allows estimation of non-breast milk water intake, and the mother's body composition (Haisma et al, 2003).

An oral dose of deuterium oxide is given to the mother and urine or saliva samples are collected pre-dose and post-dose over a period of 14 days (Davidsson, 2005) for the mother (days 1, 4 and 14) and infant (days 1, 3, 4, 13 and 14). The total volume of breast milk consumed by the infant over a period of 14 days is determined by measuring the appearance of deuterium in the infant's urine or saliva (Davidsson, 2005) and the disappearance of deuterium in the mother's urine/saliva sample.

The enrichment of body fluids (saliva, urine or serum) after the deuterium dose equilibrates with the body water pool can be used to calculate the maternal dilution space (N) hence total body water (TBW) by back extrapolation method based on isotope ratio mass spectrometry (Wells et al, 2005) based on equation 2-1:

$$N = \frac{TA}{a}\left(\frac{E_a - E_T}{E_S - E_P}\right) \quad \text{..... Equation 2-1}$$

where A is the dose of isotope in grams, a is the portion of the dose in grams retained for mass spectrometer analysis, T is the amount of tap water in which the portion a is diluted before analysis, and E_a, E_T, E_p, and E_s are the isotopic enrichments in delta units of the portion of dose, the tap water used, the pre-dose sample, and the post-dose sample, respectively. Delta units express isotopic enrichment relative to a standard, in this case Vienna-Standard Mean Ocean Water (VSMOW).

This technique has been validated (Infante et al, 1991; Wong et al 1990), and successfully applied in field conditions in developing countries (Haisma et al; 2003; Butte et al, 1993; Salazar et al, 2000; Ettyang et al, 2004; Ettyang et al, 2005). There is a dearth of data on body composition and breast milk intake in infants 6 - 12 months old.

3 Rationale and Objectives

The period of complementary feeding determines a child's growth and survival in the short term and has long term implications for psycho-motor development and work capacity in later life. Even though the promotion of breast feeding has received much attention in the past decade, similar success has not been realised in the efforts to provide safe and nutritionally adequate complementary foods in many developing countries (WHO, 2003a; Pelto, 2003).

Poor quality complementary foods, characterised by high dietary bulk and low energy and nutrient density, and inappropriate feeding practices have been identified among the major causes of malnutrition in young children in developing countries (WHO, 2003a). In many developing countries, complementary foods are introduced too early or too late and the quality and quantity of the foods are insufficient leading to a great risk of nutritional deficiencies during the second half of infancy (Pelto et al, 2003).

Food selection and preparation practices which affect food safety, nutrient density and feeding practices and maternal characteristics such as education, and socio-economic status have been identified as inte.g.ral components of care-giving in relation to nutrition of young children (Pelto et al, 2003). The dearth of data on mother-infant interaction during feeding has been highlighted (Pelto et al, 2003). Provision of safe and appropriate complementary foods processed using indigenous nutrient-rich foodstuffs was emphasized at the World Health Assembly (WHO, 2002). However, the availability of high-quality, low-cost

fortified complementary foods on the commercial market is almost ne.g.ligible in the developing world (Lutter, 2003). The success in combating micronutrient deficiencies through complementary food fortification strate.g.ies in industrialized countries (Darton-Hill, 1998; Chen and Oldewage-Theron, 2002; Ramakrishnan and Yip, 2002; ILSI, 2003) may be applied in resource poor settings with relevant modifications to suit specific population needs. One example is the low prevalence of iron deficiency (5%) in industrialized countries compared to approximately 40% in developing countries (Yeung and Kwan, 2002).

The need for nutritionally adequate complementary foods is especially relevant in circumstances where the HIV prevalence is high. Infants born to HIV-infected mothers often have lower birth weights and are more prone to growth faltering than infants of HIV-non-infected mothers (Miller et al, 1993). Further, the knowledge that HIV-1 can be transmitted through breastfeeding (Nduati et al, 2000) might result in decreased initiation or duration of breastfeeding. The dilemma of HIV and breastfeeding poses an urgent challenge to address the needs of children beyond the period of exclusive breastfeeding (Piwoz et al, 2003) through the development of improved complementary or replacement foods and adoption of better feeding practices.

Traditionally used complementary foods are inadequate in iron, zinc and pyridoxine and in some populations may be deficient in riboflavin, niacin, calcium, thiamine, folate, ascorbic acid and vitamin A (Lutter and Rivera, 2003). The fact that it is difficult to meet the micronutrient needs, particularly those of iron and zinc, of infants beyond 6 months of age, especially non-breast infants,

without food fortification is now recognized (Dewey et al, 2004; Ruel et al, 2004). There is an urgent need to explore the production of multi-micronutrient fortified complementary foods based on locally produce and widely used foods stuffs such as beans and groundnuts.

Work on the development of nutritious weaning food blends based on locally available Zambian le.g.umes and cereals has been published (Hayes et al, 1995). Evaluating complementary foods for acceptability is useful in their optimization before they are introduced into the market (Mensa-Wilmot et al, 2001) or used for intervention programmes. This is more relevant in cases, like the present study, where ingredients that are not commonly used, such α-amylase and vitamin-mineral fortificants, are used to modify the foods.

Complementary foods may partially displace breast milk (Haisma et al, 2003; Haisma et al, 2004) and may also interfere with the absorption of nutrients in breast milk (Brown et al, 1995) thereby producing a greater likelihood of nutrient deficiencies. Haisma et al (2003) working in Brazil found that exclusively breastfed infants had significantly greater breast milk intake than partially breastfed infants (a difference of 203 g/d) at 4 months of age. A recent study from India (Bajaj et al, 2005) reported significant displacement of breast milk (121 g/d) following short term oil supplementation of complementary foods in breastfed infants 6-10 months old. Another study from Bangladesh (Kimmons et al, 2004) found that although greater intakes of complementary foods were associated with higher total energy intake, micronutrient intake was inadequate due the low micronutrient density of the complementary foods consumed and the partial

displacement of breast milk. Multi-micronutrient fortified, nutrient-dense complementary foods may partially displace less nutritious traditional complementary foods resulting in increased energy and nutrient intake. It is thus important to accurately measure the amounts of complementary foods consumed and breast milk intake of infants and young children (Haisma et al, 2004).

3.1 Objectives

The objectives of the study were: 1) assess the complementary feeding practices of mothers and the nutrient intakes of infants 6-18 months old; 2) develop and evaluate the acceptability of α-amylase-modified complementary food based on locally available cereals and le.g.umes; 3) assess the cost of an industrially processed α-amylase-modified multi-micronutrient fortified complementary food based on locally available cereals and le.g.umes; 4) assess through a randomized controlled trial the effect of the α-amylase-modified multi-micronutrient fortified complementary food on growth and haemoglobin concentration of 9-month old infants and to generate data on nutrient and breast milk intake.

3.2 Study site

The study was based at Chilenje clinic (Figure 3-1), Lusaka, Zambia (Figure 3-2). The site was chosen since the research group has been working there for 3 years on the Breastfeeding and Postpartum Health Project and had developed links with the clinic and community. Chilenje is a middle income urban area where most households have running water and flush toilets. A middle income urban area was chosen in line with the WHO Global Strate.g.y on Infant and Young Child

Feeding which notes that industrially processed complementary foods are an option for mothers who can afford them and have the knowledge and facilities to safely prepare and feed them (WHO, 2002). Secondly, the problems of micronutrient deficiency affect a much larger proportion of the population than those who have evident clinical symptoms (Maberly et al., 1998). More sensitive biological sample testing in populations show evidence (Maberly et al., 1998) that infants and children are affected in both urban and rural settings. Our preliminary results on micronutrient intake of Chilenje infants 6-9 months old show that these children are receiving only 33% of the recommended daily allowance for absorbable iron for this age range. It is necessary, therefore, to evaluate the effectiveness of multi-micronutrient fortification of complementary foods not only in poor rural areas, but also in low-to-middle income urban areas like Chilenje.

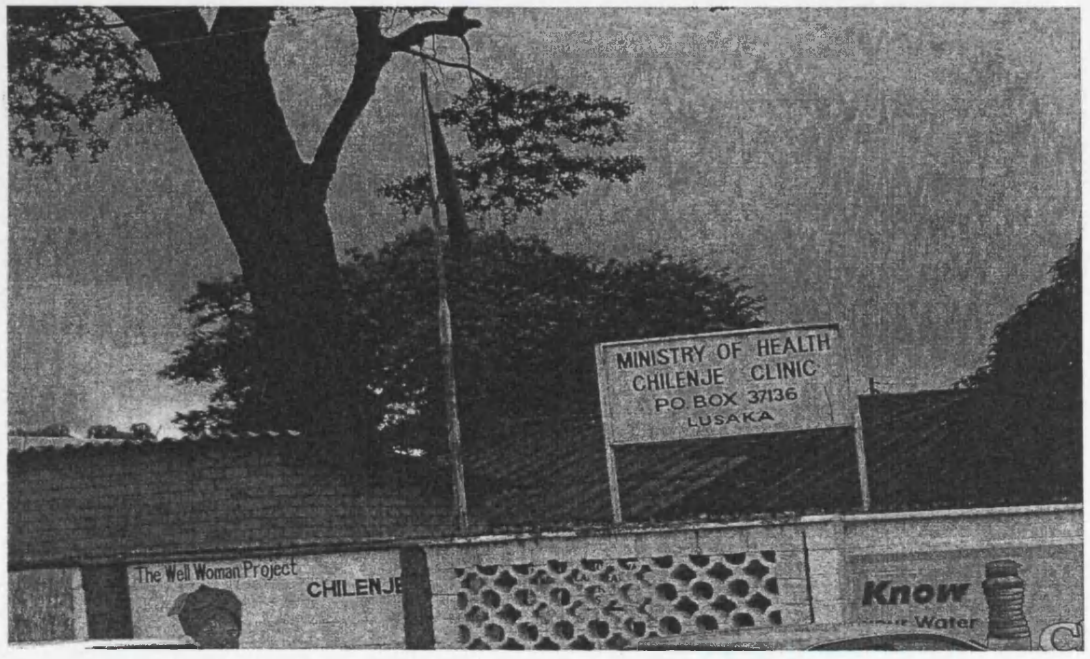


Figure 3-1 Chilenje clinic

Figure 3-2 Map of Zambia

4 Methods

4.1 Introduction

This was a collaborative study among the Institute of Child Health (London, UK), Lusaka District Health Management Team (Lusaka, Zambia), National Institute for Scientific and Industrial Research (Lusaka, Zambia), University Teaching Hospital (Lusaka, Zambia), London School of Hygiene and Tropical Medicine (UK) and Quality Commodities Limited (Lusaka, Zambia).

4.2 Overall study design

The study consisted of three successive stages. Figure 4-1 presents the main study stages and successive activities undertaken between May 2003 and March 2005. The first stage was designed to assess the complementary feeding practices and nutrient intake from traditional complementary foods of children aged 6-18 months using a combination of qualitative and quantitative methods. The second stage was based on findings from the first stage and involved two phases namely, 1) development and assessment of the acceptability of an improved complementary food based on roasting and addition of α-amylase, and 2) industrial processing of the developed complementary food with food fortification and α-amylase addition and determination of its storage stability and cost. The third stage involved a randomized controlled trial of the effect of the α-amylase-treated fortified complementary food developed on growth, haemoglobin concentration and nutrient intake of infants 6-9 months old and assessment of breast milk intake by deuterium-dose-to-the-mother technique.

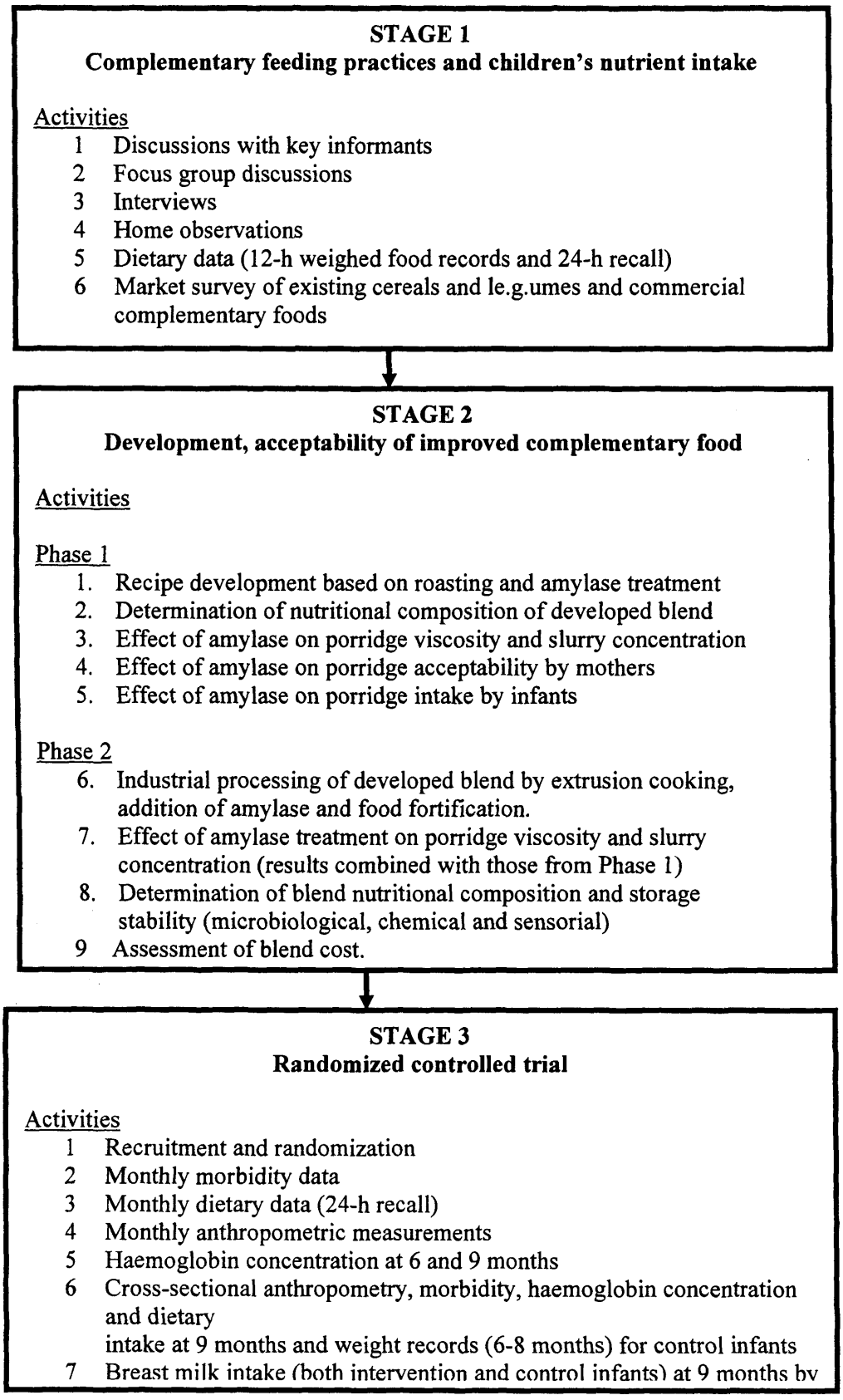


Figure 4-1 Main study stages and activities undertaken

4.2.1 Stage 1: Complementary feeding practices and nutrient intake of children 6-18 months old

Qualitative data were obtained using three main techniques namely, focus group discussions, interviews and home observations in order to allow for the triangulation of findings (Pope and Mays, 1995). A qualitative approach was chosen to obtain information on knowledge, attitude and complementary feeding practices that could not be measured by standard quantitative methods (Pope and Mays, 1995). Secondly, findings obtained by qualitative methods were used as the basis for the subsequent stage of complementary food development. Quantitative data were obtained by 24-h dietary recall and 12-h weighed food record. All participants gave written consent to attend the focus group discussions and had the ability to speak English. The requirement that participants spoke English was not a major source of bias as English is the official language in Zambia and schooling is in English.

4.2.1.1 Focus group discussions

Three focus group discussion (FGD) sessions were held at Chilenje clinic based on the same FGD guide (Box 4-1) with the help of two trained assistants (nutritionist and nurse) to assess current complementary feeding practices, attitudes and beliefs that would influence any nutritional intervention based on the provision of improved complementary foods. The three groups attending the discussions were: 1) 9 health workers from Chilenje clinic; 2) 7 mothers from Chilenje who had children aged 6-18 months 3) 8 fathers from Chilenje who had infants and children aged 6-18 months. These three groups were purposely chosen because they had direct involvement in child care and complementary feeding

decision making process. However, it is possible that the group chosen was not representatitive of the entire population. Individual mothers, nurses and fathers were invited by one of the assistants who had good rapport with the Chilenje community based on her involvement in a previous project by our group. Focus group themes were explored using the same question guide for all the three focus groups discussion sessions. Proceedings of each discussion were recorded in note books by VO and one assistant and on audio cassette which was later transcribed. Generated themes were used to design the semi-structured questionnaire for interviews.

Box 4-1 Focus group discussions guideline

1. Locally available foodstuffs used for feeding young children
2. Prices of foodstuffs in local markets and shops
3. Mostly used ingredients for preparation of food for young children
4. Introduction of foods other than breast milk
   - cessation of breastfeeding
   - factors determining introduction of complementary foods
   - frequency of breastfeeding
   - frequency of feeding other foods
5. Factors determining the choice and use of foods for feeding children
6. Preparation of food for young children
   - Who prepares food
   - Frequency of food preparation
   - Fuel use and cost
7. Commercially available complementary foods

4.2.1.2 Interviews

Table 4-1 shows selected demographic characteristics of the 34 interview subjects. Most (62%) of the households had 2-3 children and 32% of the households had another child aged below 5 years.

Table 4-1 Selected demographic characteristics of the mothers interviewed (n = 34).

Variable	N (%)
Child's sex	
Boys	18 (53)
Girls	16 (47)
Child's age (mo)	
6–8	11 (32)
9–11	10 (30)
12-18	13 (38)
Mother's age (years)	26 (SD 7)
Marital status	
Married	26 (76)
Single	8 (24)
Mother's education	
Primary (1-7 grades)	8 (23)
Secondary (8-12 grades)	19 (56)
Tertiary colle.g.e	7 (21)
Mother's occupation	
Salaried employed	5 (15)
Self-employed	4 (12)
Housewife	22 (65)
Unemployed	3 (8)

A total of 34 mothers of infants and children 6-18 months old were interviewed by VO and two assistants using a semi-structured questionnaire (Appendix 9-1) that had been pre-tested at Chilenje clinic with three mothers of infants and children of the same age group (6-18 months). Participants were invited by the two trained assistants as they brought their children for growth monitoring at the clinic. The

main inclusion criteria were that the mother was able to speak in English and gave informed consent.

The purpose of the interviews was to assess the availability and factors affecting the use of cereals and le.g.umes and commercial complementary foods in addition to complementary feeding practices, knowledge, beliefs and attitudes. The questionnaire was divided into three main sections concerning household characteristics, food availability, and infant feeding and care practices. The third section was divided into four subsections namely, breastfeeding practices, infant care and time availability, complementary feeding practices, and food preparation and handling. All mothers were asked after the interviews if they would give consent for home visits after two weeks; 20 mothers gave written consent: 7 with infants 6-8 months, 6 with infants 9-11 months and 7 with children 12-18 months.

4.2.1.3 Home observations

The 20 mothers were observed at home for 12-hours during day light by VO accompanied by a female nurse. Although this approach provides first hand information, it is intrusive (Creswell, 1994) and the results may be biased due to change of behaviour by the subject in the presence of an observer (Gibson and Ferguson, 1998; Gibson and Ferguson, 1999). Mothers were asked to continue with their normal routines while VOs determined their actual food preparation and handling behaviour and how they fed their children.

4.2.1.3.1 Macronutrients and food solids intake by children from traditional diets

Average breast milk intake (565 g/d) was assumed based on average breast milk energy intakes in developing countries of 413 kcal and 379 kcal, respectively for infants 6-8 months and 9-11 months old and, 346 kcal for young children 12-23 months old (Dewey and Brown, 2003). Breast milk results obtained in stage 3 of the current study were not used for calculations since they are limited to infants 9 months of age.

Dietary intake was determined by 12-h weighed food record for the day of the visit and 24-h recall for the day preceding the observation. These data were used to calculate the daily intake of energy, protein, fat, food solids, iron, calcium and the energy densities of traditionally used porridges based on a Zambian food composition table (National Food & Nutrition Commission, 1997). Data for iron absorption from complementary foods and breast milk and the recommended dietary allowance (RDA) for absorbable iron were taken from the current WHO estimates (Lynch and Stoltzfus, 2003). Low iron absorption (2.4 – 6%) from maize-based diets was assumed. Daily amounts of absorbable iron obtained from breast milk were assumed to be 0.11mg/day and 0.09 mg/day for children 6-12 months and 13-18 months, respectively (Lynch and Stoltzfus, 2003). The daily intake of calcium from breast milk was assumed to be 130 mg, the estimate for children 6-23 months old, and calcium retention from breast milk and solid foods was assumed to be 50% and 20-25%, respectively (Lutter and Dewey, 2003). Energy and absorbable iron obtained from 12-h weighed food record and 24-h recall of the day preceding the observation were compared. A total of 10 and 9

porridge recipes obtained by 12-h weighed food record and 24-h recall, respectively were compared.

4.2.2 Development, acceptability and costing of improved complementary food

4.2.2.1 Recipe development (roasted α-amylase-treated blend)

After interaction with mothers and assessment of market price and availability of raw grains and le.g.umes, an affordable mix was designed based on locally produced foodstuffs. Three preliminary recipes labelled 1, 2 and 3 were designed to meet the nutritional needs of infants children aged 6 – 24 months (Lutter and Dewey, 2003) using four main ingredients, namely, maize (*Zea maiz*), common beans (*Phaseolus vulgaris*), bambaranuts (*Vorandzea subterranean*) and groundnuts (*Arachis hypogea*) in the ratios 65:15:5:15, 65:15:10:10 and 70:10:10:10, respectively. Table 4-2 gives the nutritional composition of maize, beans, bambaranuts and groundnuts. These ingredients were chosen because they are affordable, widely used, and available in Lusaka throughout the year. All ingredients were purchased from Lusaka City Market. Ingredients were washed, drained and roasted in a kitchen oven at 150 °C for 25-30 minutes to a golden brown colour. The roasted ingredients were cooled before hammer milling. Table 4-3 gives the calculated energy composition and estimated price (US$/kg) of the three preliminary recipes. Recipe 1 was most accepted by mothers and was adopted as the working blend.

Table 4-2 Nutritional composition per 100g of maize, beans, bambaranuts and groundnuts

	Ingredient			
	Maize[a]	Beans[a]	Bambaranut[b]	Groundnut[c]
Protein (g)	9.1	22	22.5	25
Carbohydrate (g)	75.3	61.4	55.8	8.6
Fat (g)	4.4	1.1	2.9	45
Energy (Kcal)	377.2	343.5	339.3	539.4
Thiamine (mg)	0.44	0.56	0.61	0.79
Riboflavin (mg)	0.13	0.2	0.21	0.07
Niacin (mg)	1.9	1.9	1.5	9.5
Pyridoxin (mg)	60	0.4	0.4	0.4
Folic acid (μg)	10.1	410	385	76
Pantothenic acid (mg)	0.6	0.7	0.8	
Iron (mg)	2.4	7.4	7.4	1.7
Zinc (mg)		2.9	3.5	2.4
Calcium (mg)	18	150	148.8	75

[a] National Food Nutrtition Commission, 1997; [b] Nwokolo, 1996a; [c] Nwokolo, 1996b; [d]Higgs, 2003

Table 4-3 Estimated energy composition, Protein Efficiency Ration (PER), net protein utilization (NPU) and basic price of three preliminary recipes compared with reference values.

	Recipe (Ingredient ratio)[1]			
	65:15:5:15	65:15:10:10	70:10:10:10	Reference value[2]
Protein (g/100kcal)	3.6	3.6	3.5	≥ 2.5
Fat (g/100kcal)	2.5	2.0	2.1	$\geq 2, \leq 6$
% protein energy	14.30	14.53	13.80	6 -10
% CHO energy	63.1	67.2	67.6	62-70
% fat energy	22.6	18.3	18.6	24-28
PER	3.0	3.0	2.9	NA
NPU[3]	78	78	75	NA
Price(USD) per Kg	0.59	0.59	0.58	NA

[1] maize:beans:bambaranuts:groundnuts (%w/w) ratio;
[2] estimated needs of children 6-18 months taken from Lutter and Dewey, 2003.
[3]The NPUs observed in the current study are higher than the estimated average value, 69.1 (SD 12) for 12 West African complementary foods containing groundnuts, cereals (maize, millet or sorghum) and another legume (Onofiok and Nnanyelugo, 1998).

4.2.2.2 Effect of α-amylase on porridge viscosity and slurry concentration

To determine the optimum level of α-amylase that would result in mother-acceptable viscosity the commercial α-amylase preparation was applied at different concentrations (% w/w of dry flour): 0.01%, 0.02%, 0.03% and 0.04%. α-amylase was premixed with dry flour before porridge slurry was made. Porridge slurry containing 20% flour (w/v) was prepared and stirred for 2-3 minutes to allow for reaction before being brought to boil at 70 °C. The porridge was cooled in a water bath to 45 °C before measurement of viscosity using Brookfield Viscometer (spindle #3 at 50 rpm). This temperature was chosen for measurement of viscosity since starch gelatinization be.g.ins at 45 °C (Tester et al, 2004) and below this point porridge viscosity is likely to increase sharply due to starch retrogradation. To determine the influence of porridge flour concentration on the

reduction of porridge viscosity by α-amylase (0.04% w/w), porridge slurries were prepared to have different flour amounts (% w/v): 9%, 13%, 17% and 20%. The viscosities of traditional porridges were measured after cooking the porridges based on recipes, containing various combinations of ingredients (maize meal, groundnuts, sugar, cooking oil, soya flour, milk), that were previously determined by 12-h home observations. Porridge viscosity was determined as described in section 4.2.6.1.

4.2.2.3 α-amylase-treated roasted porridge acceptability assessment

The three recipes were subjected to acceptability evaluation in terms of colour, smell, taste, thickness and general preference by 18 mothers at Chilenje clinic. Initially we piloted a porridge acceptability test using a conventional 9-point hedonic scale, but most mothers found it difficult to understand. It was necessary to develop a simple acceptability evaluation tool (Appendix 9-2). The new tool combined smiley faces and a traffic lights concept and had 3 points represented as follows: 1 (green smiling face) = I like it, 2 (orange straight face) = I don't know, and 3 (red sad face) = I don't like it.

4.2.2.4 Effect of α-amylase on infants' intake of roasted porridge

Eighteen mothers were provided with 140 g coded sample of flour with α-amylase and another 140 g of flour without α-amylase to take home and prepare porridge using their usual way of porridge preparation. Mothers were provided with a separate form for each flour and were instructed to record the amount in cup units of porridge prepared, amount served and actual amount eaten by the infant and if the child had diarrhea or unusual behaviour after consuming the provided porridge. This set of data was used to calculate the percentage of porridge serving

consumed by the infant and to compare the consumption of α-amylase treated maize-beans-bambaranuts-groundnuts blend, non-α-amylase-treated blend and the traditional porridges. It is noted that this was a semi-quantitative method and may have resulted in some error in the estimation of infants' porridge intake.

4.2.2.5 Industrial production of α-amylase-treated fortified blend

Recipe 1 (65:15:5:15) accepted by mothers from the recipe development stage was adopted for the next phase of this work. Maize, *kabulangeti* beans were supplied by Quality Commodities Limited. Groundnuts and bambaranuts were purchased from the Lusaka city council market. Commercial preparation of α-amylase was purchased from Equatorial Food Ingredients, Lusaka.

Vitamin-mineral pre-mix, except calcium and phosphorus (as tricalcium phosphate) was donated by DSM Nutritional Products, South Africa. It was not possible to obtain the exact premix composition from DSM likely due to the fact that the calculations of mineral and vitamin content of the blends were done by the study group. It is noted that although premix composition data from DSM Nutritional Products is not available, the mineral and vitamin composition of the blends were based on current recommendations for infants 6-11 months old (Lutter and Dewey, 2003) taking safety and potential cooking losses into account. Calcium and phosphorus (as tricalcium phosphate) were purchased from Nitada Africa PTY, South Africa. The blend comprised 65% maize, 15% *kabulangeti* beans, 5% bambaranuts and 15% groundnuts. These main ingredients were processed at Quality Commodities Limited, Lusaka in two batches of 2000 kg each at 3 months by extrusion cooking as shown in Figure 4-2 using single screw extruder (Single screw X20, 3.25 inch barell, 220-1000 kg/h, Wenger

Manufacturing Inc, Sabetha, USA). Each batch was divided into two equal halves. Both halves were fortified with vitamins and minerals at levels taking into account infant's daily nutrient needs based on an assumption of low breast milk intake (Dewey and Brown, 2003) and overages to account for storage and cooking losses. Iron was added as electrolytic iron, zinc as zinc oxide. Minerals and vitamin premix, tricalcium phospahate and α-amylase were added to the blends after extrusion cooking to avoid vitamin losses and α-amylase inactivation at high processing temperatures. Table 4-4 presents the nutrient amounts in the fortified maize-beans-groundnuts-bambaranuts blend with or without amylase. The figures were based on a daily complementary food solids intake of 50 g (Lutter and Dewey, 2003) and a 40% increment in solids intake with the addition of amylase (den Besten et al, 1998 and our preliminary results). Due to lack of funds and limited local capacity for mineral and vitamin assays, it was not possible to obtain quality data on the mineral vitamin content of the fortified study blends. However, this limitation has been corrected for by measuring iron, zinc and vitamin A in the fortified blends used in the follow-up study, Chilnje Infant Growth Nutrition and Infection Study (CIGNIS) that is underway in the same population.

α-amylase (0.04% w/w of flour) was added during vitamin-mineral premix mixing to one half. The two fortified blend treatments were named according to amylase-treatment i.e. Chilenje Baby Mix [CBM (non-amylase treated fortified blend)] and Chilenje Baby Mix with amylase [CBMA (amylase-treated fortitified blend)]. To assess acceptability by mothers and infants of the extrusion cooked blend, mothers were informally asked by study assistants when they came for monthly porridge

supply (see randomized controlled trial section below) to describe how they liked the blend in terms of ease of cooking, colour, taste and how much the child ate. The influence of porridge flour concentration on the reduction of porridge viscosity by α-amylase (0.04% w/w) was determined as described above (section 4.2.4.1) at 8%, 12%, 16% and 20% (w/v) slurry concentrations.

Table 4-4 Nutrient amounts in the fortified maize-beans-groundnuts-bambaranuts blend with or without amylase

Nutrient	Estimated needs/day[1]	Amount in 240 ml breast milk/day[1]	Daily intake from 50g of CBM[2]	Daily intake from 70g CBMA[3]
Vitamin A (μg)	400	84	350	490
Vitamin C (mg)	30	24	100	140
Vitamin D (μg)	5	0.2	5	7
Thiamin (μg)	300	50	450	630
Riboflavin (μg)	400	84	560	784
Niacin (mg)	4	0.4	6	8.4
Pyridoxine	300	31	430	602
Folate (μg)	80	20	110	154
Pantothenic acid (mg)	1.8	0.5	2	2.8
Calcium (mg)	400	60	350	490
Phosphorus (mg)	275	26	275	385
Magnesium (mg)	54	7	50	70
Iron (mg)	20.9	0.04	20.9	29
Zinc (mg)	5	0.1	5	7
Copper (μg)	200	40	200	280
Manganese (μg)	600	0.7	600	840
Selenium (μg)	10	-	10	14

[1] Dewey and Brown, 2003;

[2] estimated intake taken from Lutter and Dewey, 2003;

[3] amylase treatment assumed to result in 40% increment in complementary food solids intake (den Besten et al, 1998 and our preliminary results).

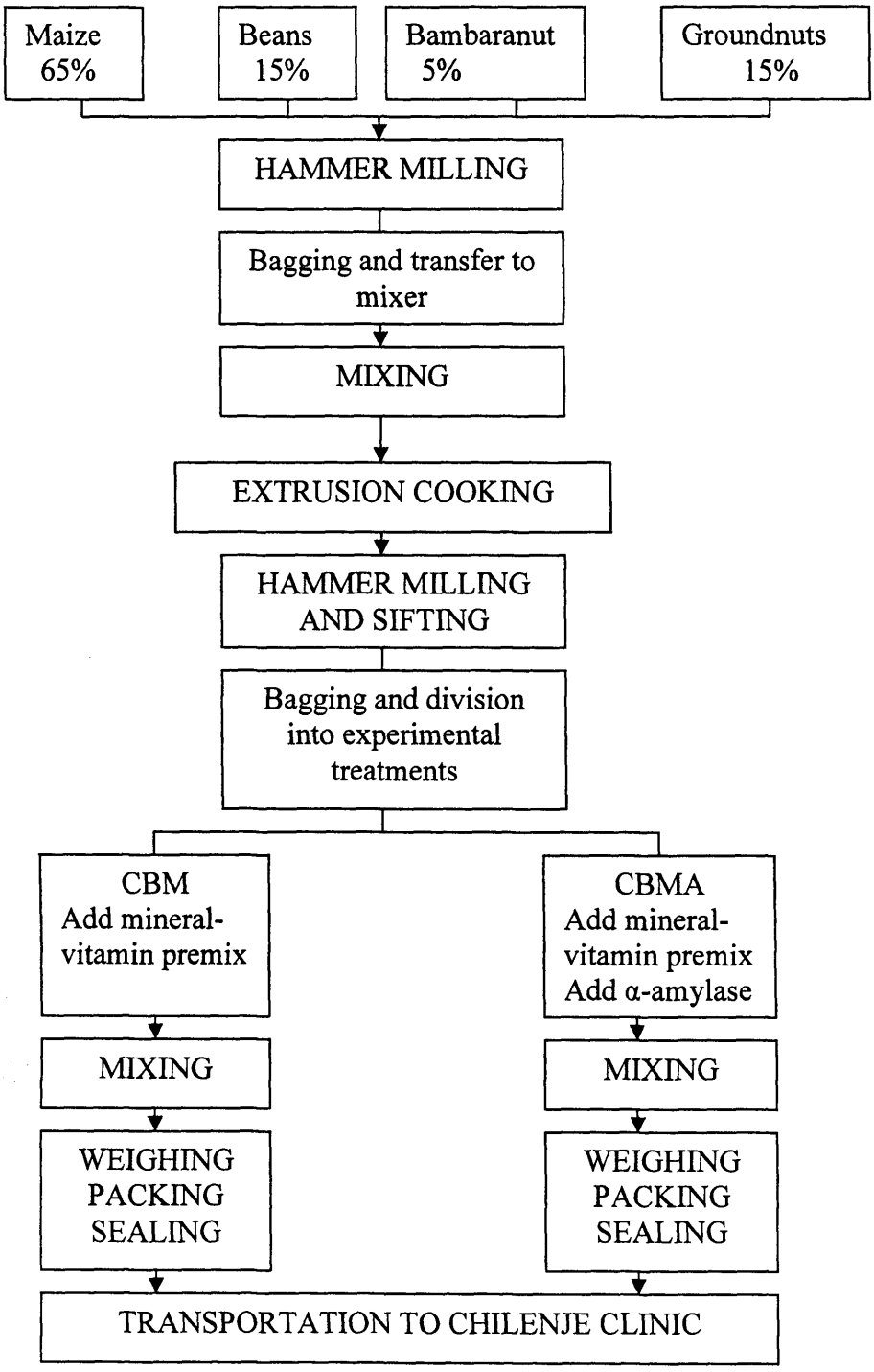


Figure 4-2 Industrial processing of complementary food by extrusion cooking[1].

[1]Micronutrient and α-amylase were added to the blends after extrusion cooking to avoid vitamin losses and enzyme inactivation at high temperature.

4.2.2.5.1 Food analysis

VO was involved in all of the food analyses procedures described except in aflatoxin analysis in which, due to restrictions at the contracted company, he visited the laboratory and observed all the equipment and procedures.

Four samples were taken at 10 minute intervals during processing at each of the following stages: 1) first mixing after hammer milling, 2) milling and sifting after extrusion cooking, 3) addition and mixing in of α-amylase and mineral-vitamin premix. The four samples from each processing stage were later mixed into one large sample from which two samples were drawn for the determination of viscosity, proximate composition, aflatoxins and storage stability.

4.2.2.5.2 Proximate composition and viscosity

Proximate composition (moisture, crude protein, crude fat, crude fibre, total ash, and carbohydrate) and viscosity of the maize-bean blend were determined at the Department of Food Technology, University of Zambia using standard methods (AOAC, 1995).

Briefly, protein was estimated by determining the total nitrogen using the Kjeldahl method. Protein was calculated using the formula N x 6.25. Fat content was determined by the Soxhlet extraction method. Ash content was estimated by charring samples in a furnace and gravimetrical quantification. Moisture content was determined by gravimetry upon drying in an air oven to constant weight at 100 °C.

Carbohydrates were calculated as % carbohydrates = 100%- (%Protein + % Fat + % Ash + % Moisture content). Fibre was estimated by treating samples with boiling acid detergent solution to dissolve the protein, carbohydrate, and ash. Fat and pigments were washed off using acetone. The crude fibre was determined gravimetrically.

Porridge viscosity was determined according to the method of Hayes et al. (1995). A 25 g of flour sample were stirred in 100 mL water at 70 °C in a 400 mL beaker. Any lumps were mashed using a spatula. Viscosity was measured at 45 °C using a Brookfield RVT viscometer (spindle #3 at 50 rpm). This temperature was chosen for measurement of viscosity since starch gelatinization be.g.ins at 45 °C (Tester et al, 2004) and below this point porridge viscosity is likely to increase sharply due to starch retrogradation.

4.2.2.5.3 Storage stability and aflatoxin content of blend

The storage stability of extrusion cooked α-amylase-treated multi-micronutrient fortified blends was assessed after storage at room temperature for 2 weeks and 6 months, respectively. Six blend samples namely, 1) 6-month old non-amylase-treated-non-fortified, 2) 2-week old non-amylase-treated-non-fortified, 3) 6-month old amylase-treated-fortified, 4) 2-week old amylase-treated-fortified, 5) 6-month old non-amylase-treated-fortified, and 6) 2-week old non-amylase-treated-fortified were assessed in duplicate for shelf-life based on 1) sensory evaluation by a panel of 18 trained panelists, 2) peroxide value, 3) water activity and 4) microbial load.

4.2.2.5.4 Microbial load

The microbiological load of a food product indicates food hygiene and its suitability for human consumption.

4.2.2.5.4.1 Total viable count, E. coli and yeasts and moulds

Flour samples were analyzed for total viable count, E. coli, and yeasts and moulds using standard methods (AOAC, 1995) at the Department of Food Technology, University of Zambia. Total viable count was determined by the pour plate method using Pour Count Agar with incubation at 37 °C for 48 h (Fang et al., 2002). E. coli was determined by the most probable number (MPN) technique (AOAC, 1995) using lauryl sulphate tryptose (LST) broth incubated at 37 °C for 48 h. Positive LST tubes were inoculated into EC broth and incubated at 44.5 °C for 48 h. Positive EC tubes were inoculated into tryptone water (TW) media and incubated at 44.5 °C for 48 h. To confirm E. coli, indole test was performed on the TW tubes by taking the culture media and adding 0.2 ml of dimethylaminobenzaldehyde, looking for the development of red colour that indicated positive indole reaction (Cakir et al., 2001).

4.2.2.5.4.2 Aflatoxins in suspect groundnut kernels and extrusion cooked blend

Clean groundnuts resulting from sorting were assumed to contain ne.g.ligible aflatoxin levels according to previously reported values. Table 4-5 shows published aflatoxin levels in stored groundnuts (without sorting), clean groundnuts (with or without sorting) and mouldy or damaged groundnut kernels.

Samples of visibly contaminated groundnut kernels and extrusion cooked blend were analyzed for aflatoxins B_1, B_2, G_1, G_2 at Cheetah Zambia Limited. Twenty five grams of ground sample and 5 g sodium chloride were weighed into a blender jar and mixed with 100ml methanol: water (80:20 v/v), blended at high speed for 1 minute. The extract was filtered using 24 μm (Whatman 1) filter paper. The filtrate was collected in a 100 ml Duran bottle.

10 ml of the filtrate was pipetted into a clean 100 ml volumetric flask and diluted with 40 ml HPLC grade filtered water, vortexed then filtered through 11 μm (Whatman 1) filter paper. Four millilitres of filtrate was passed through an affinity column via a 10 ml syringe at a rate of 2 drops per second. The affinity column was washed twice with 10 ml methanol:water solution (20:80 w/w) at a rate of 2 drops per second. The affinity column was eluted by passing 1 ml of HPLC grade methanol at a rate of 1-2 drops per second. The eluate was collected in a 10 ml glass cuvette, diluted with 1 ml HPLC grade filtered water and mixed thoroughly by vortexing. One millilitre of eluate was put in a 1 ml cuvette in readiness for HPLC determination.

Prior to sample injection, pumps and injector were purged and primed to remove all the air bubbles and equilibrated for 15 minutes. The calibration curve was developed by injecting 10μL, 20μL and 30μL of mixed aflatoxin standard. 100μL of sample was delivered to a C18, RPHPLC (3.9 x 150 mm) column (NovaPak) via an auto injector by a binary pump (GenTech Scientific, HP 1100 HPLC System). The column effluent was mixed in a Kobra cell (100 uA) (Rhone Diagnostics) with a derivatisation solution that consisted of 550 ml HPLC grade

filtered water, 119 mg potassium bromide and 87.5 μL nitric acid which had been filtered through 0.22 μm filter paper before delivery to a fluorescence detector (Gain 100) set at 362 nm extinction and 425 nm emission coupled to a PC with Bus SAT/IN module.

Table 4-5 Aflatoxin levels in stored, clean/undamaged and mouldy/damaged groundnut kernels

	Aflatoxin level/range (μg/kg)	Country	Reference
Stored groundnuts	43-1099	Brazil	Freitas and Brigido, 1998
	12.5 – 528	Benin & Togo	E.g.al et al, 2005
	300	Philippines	Galvez et al, 2003
Clean/undamaged kernels	0.1-12.2	Ghana	Awuah and Kpodo, 1996
	< 15	Philippines	Galvez et al, 2003
Visibly damaged/mouldy kernels	5.7-22,168	Ghana	Awuah and Kpodo, 1996

4.2.2.5.5 Chemical and sensorial stability

4.2.2.5.5.1 Water activity

Processed blend water activity (a_w), a measure of water availability for chemical and biological reactions, was read using a_w meter (AquaLab Series 3).

4.2.2.5.5.2 Peroxide value

Since the blends were processed from groundnuts characterized by highly unsaturated fatty acids (Higgs, 2003), lipid oxidation during storage was likely, especially in the presence of minerals such as iron and zinc (Rosado et al, 2005). Peroxide value is an indicator of lipid oxidation (Rosado et al, 2005).

Peroxide value was determined in Chilenje Baby Mix based on standard methods (AOAC, 1995) as described previously (Bakirci et al, 2002) to determine the level of rancidity after 2 weeks and 6 months of storage, respectively at ambient temperature. Briefly, about 3 g of flour was weighed in an Erlenmeyer flask. 10 mL of chloroform was added, followed by 15 mL acetic acid and 1 mL saturated potassium iodide (KI) and mixed for 1 minute. The mixture was left in the dark at room temperature for 5 minutes. 75 mL distilled water was added and mixed thoroughly. 1 mL of 1% starch solution indicator was added before titration with 0.1 N sodium thiosulphate ($Na_2S_2O_3$) to a clear end point. The peroxide value was calculated as:

$$PV = [(V_1-V_0)*N]/M \quad \text{..... Equation 4-1}$$

Where V_1 is mL $Na_2S_2O_3$ used for titration, V_0 mL $Na_2S_2O_3$ used for blank, N is the normality of $Na_2S_2O_3$ and M is the amount of sample (g).

4.2.2.5.6 Sensory evaluation

18 panelists comprising students and staff from the Faculty of Agriculture, University of Zambia evaluated the six blend samples outlined above for aroma, texture and appearance based on a 9-point hedonic scale as follows: 1 = like extremely, 2 = like very much, 3 = like much, 4 = like a little, 5 = neither like nor dislike, 6 = dislike a little, 7 = dislike much, 8 = dislike very much, 9 = dislike extremely. These panelists were part of the sensory evaluation team that had been trained for routine sensory assessment at the Department of Food Technology, Faculty of Agriculture.

4.2.2.5.7 Costing of improved complementary food

The price per kilogramme of Chilenje Baby Mix was determined based on the costs of main ingredients, freight, labour and equipment costs. A profit margin of 15% was assumed based on a figure used by Quality Commodities Limited.

4.2.3 Randomised controlled trial

4.2.3.1 Hypotheses

4.2.3.1.1 Primary hypothesis

1. Infants consuming α-amylase-modified Chilenje Baby Mix have higher total micronutrient and energy intake than infants consuming traditionally used non-breast milk foods.

4.2.3.1.2 Secondary hypotheses

2. Consumption of the Chilenje Baby Mix will reduce intake of other solid foods traditionally fed to infants 6-9 months old;
3. Consumption of the Chilenje Baby Mix will reduce breast milk intake
4. Infants consuming Chilenje Baby Mix will have higher growth compared to infants consuming traditionally used non-breast milk foods;
5. Infants consuming Chilenje Baby Mix will have higher haemoglobin concentration levels compared to infants consuming traditionally used non-breast milk foods;
6. Infants consuming α-amylase-modified Chilenje Baby Mix will have higher total micronutrient and energy intake than infants consuming non-α-amylase modified Chilenje Baby Mix.

4.2.3.2 Objectives

1. Assess the effects of Chilenje Baby Mix (α-amylase-treated and non-α-amylase-treated) on macronutrient and micronutrient intake of infants 9 months old

2. Evaluate the influence of the Chilenje Baby Mix on infant growth and haemoglobin concentration.

3. Generate data on breast milk intake of infants 9 months old in Chilenje, Lusaka.

4.2.3.3 Main study outcomes

1. Growth (weight, length, body composition)
2. Energy and nutrient intake
3. Breast milk intake
4. Haemoglobin concentration

4.2.3.4 Subjects

Mothers of infants were invited to participate in the study as they brought their children to Chilenje clinic for DPT/oral polio vaccination at 5 months of age. Figure 4-3 shows recruitment in progress at the Mother and Child Health Departent, Chilenje clinic. The selection criteria for infants at 5 months of age were: mothers gave informed consent (Appendix 9-3) to 1) prepare and feed their children with the study blend; 2) attend clinic for growth monitoring 3) allow blood sampling from the infant for haemoglobin concentration measurement at the clinic. Exclusion criteria were 1) infant had evidence of chronic disease (active TB, symptomatic HIV); 2) mother did not consent. Control infants at 8 months of age were recruited one month prior to the end of the feeding programme to allow for measurements when they were 9 months together with the study group in order to avoid seasonal variation in data. The control group was recruited later since they would not receive the study blends that were expected to be of

nutritional benefit to infants in the intervention group. The provision of free complementary foods is associated with improved child growth in developing countries (WFP, 2004). The decision to recruit the control group later was also driven by advice from the local mother and child health care providers. The selection criteria at 8 months were: mothers gave informed consent to 1) attend clinic for growth indicator measurements; 2) allow blood sampling for haemoglobin concentration measurement. Exclusion criteria at 8 months included 1) infant had evidence of chronic disease (active TB, symptomatic HIV); 2) mother did not consent. Appendix 9-4 presents the questionnaire used to collect household demographic data at recruitment.

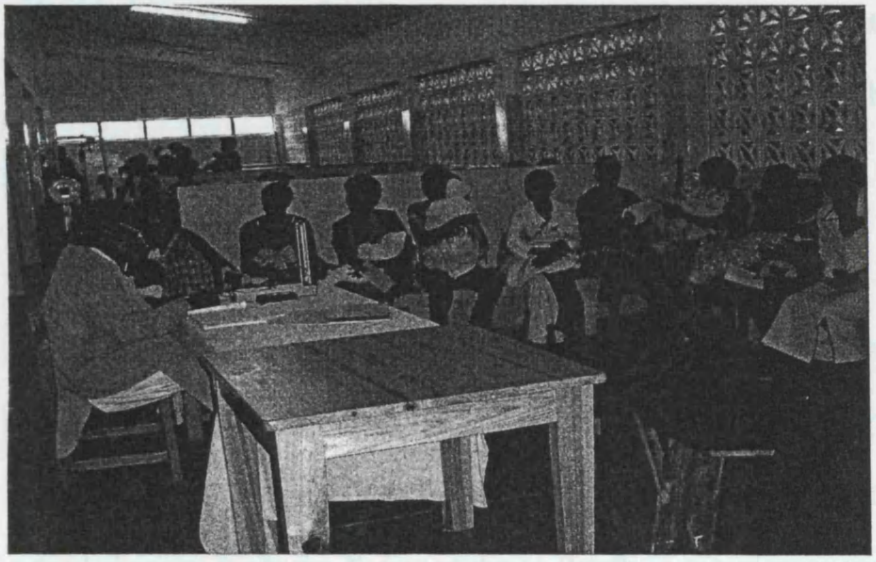


Figure 4-3 Recruitment of study subjects.

4.2.3.5 Sample size calculation

The sample size calculation was based on the primary hypothesis that infants consuming CBMA will have higher total energy and micronutrient intake than infants consuming traditional non-breast milk foods and will therefore have better

growth and micronutrient status. Since micronutrient deficiencies, especially in iron, were expected to be of more concern than energy deficiency in this population, sample size was calculated to allow for the detection of at least 5 g/L differences in hemoglobin concentration at 80% power and 5% significance. One-tailed distribution was assumed due to the fact that giving free nutrient-dense food was unlikely to decrease nutrient intake. At least 58 infants per group were needed (Figure 4-4). Based on experience from a preceding Breast Feeding and Postpartum Healh Ptroject conducted in Chilenje clinic by the study group, 15% loss to follow up was assumed. 67 infants per group were needed.

Sample size for the measurement of breast milk (25 infants per group) was based on detecting differences of 100 (SD 130) g/d in breast milk intake between groups as in a Brazilian study (Haisma et al, 2003). A small sample size for breast milk intake was partly due to the fact that stable isotope analysis based on mass spectrometry is expensive.

4.2.3.6 Preparation of study foods

Details of recipe formulation, acceptability, costing and shelf life of the study complementary foods are discussed above. Briefly, a complementary blend containing 65% maize, 15% beans, 5% bambaranuts and 15% groundnuts was industrially processed based on extrusion cooking at Quality Commodities Limited, Lusaka. The batch was divided into two equal portions after extrusion cooking and milling. Both portions were fortified with multi-micronutrients as currently recommended for infants 6-9 months old (Lutter and Dewey, 2003).

The blends were packed in 1 kg packets which were labeled with computer generated random numbers corresponding to CBM or CBMA. The list of random numbers assigned to the two blend treatments were kept away from Chilenje clinic to blind VO and assistants who distributed the blends to mothers.

4.2.3.7 Randomisation

Mother-infant pairs were individually randomised in a block design (Grimshaw et al, 2003) to receive either Chilenje Baby Mix treated with α-amylase (CBMA) or Chilenje Baby Mix without α-amylase (CBM) when the infant was 6 months old. Ten numbers (consisting of 5 numbers from either treatment) corresponding to the random number marked on the complementary food packets were placed in an envelope. Mothers were presented with envelope and asked to pick out a number. Only one assistant kept a record showing the blend number assigned to a specific mother-infant pair. A subsequent envelope was only opened when the previous one was empty. Due to ethical considerations control mother-infant pairs were measured only when the infant was 9 months old. Clinic weight records for the control infants at 6 -8 months were obtained from Lusaka District Health Management Team to enable before and after intervention comparison with the intervention groups. Each mother was given 2 kg of the same blend per month for the study infant. Data from the first phase of the study showed that about 15% of mothers in Chilenje had more than one child under the age of 3 years. 2 kg of the same blend was supplied per month for every extra child under 3 years. Infants in the control group were supplied with 4 kg (two months' supply of blend) after measurements. Figure 4-4 shows the flow diagram for recruitment and follow up of intervention infants (CBM and CBMA) from 6-9 months and control infants at

9 months of age. Figure 4-5 shows mother receiving a month's supply of Chilenje Baby mix from a study nurse.

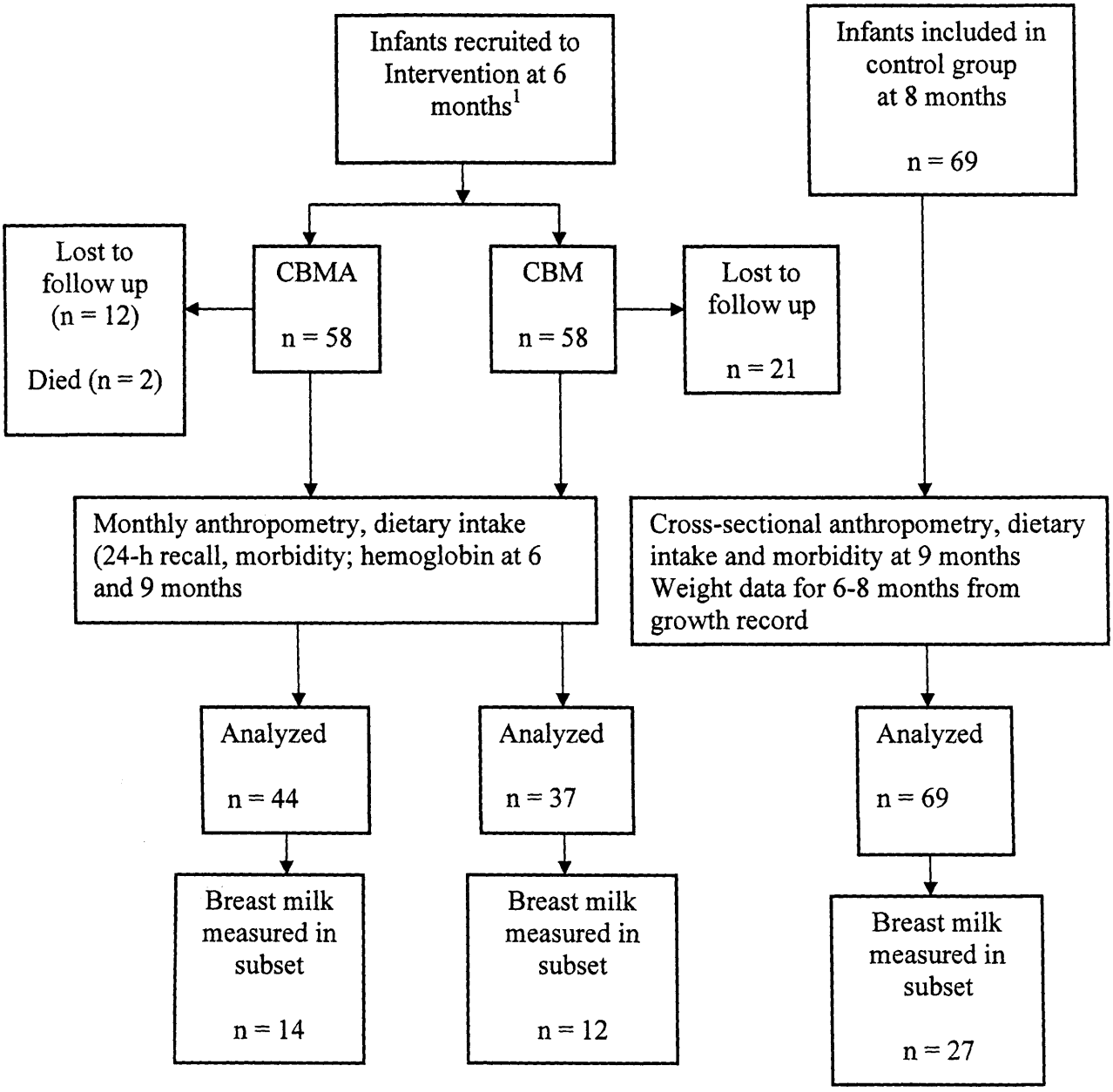


Figure 4-4 Recruitment and follow-up flow diagram for randomized trail.

[1]Although 67 infants were needed per group only 58 infants were actually recruited due to lack of time and local logistics.

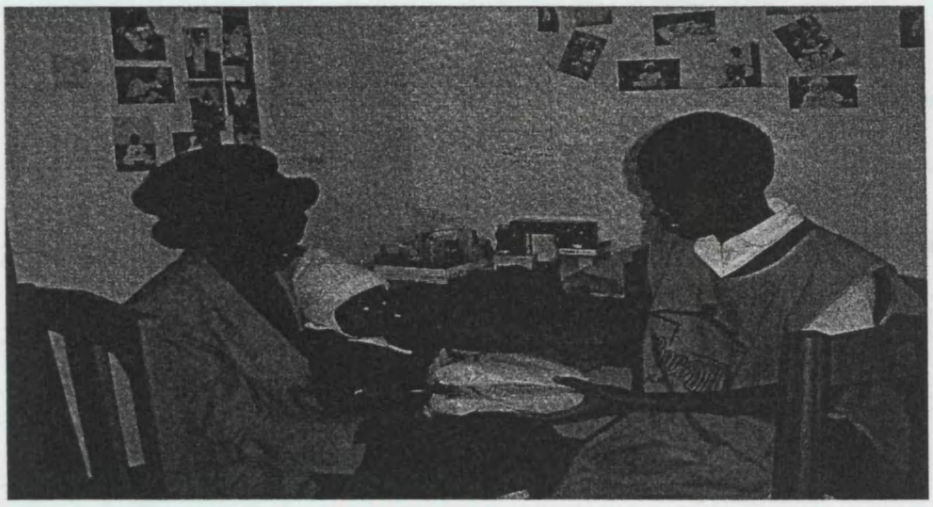


Figure 4-5 Mother receiving one month's suppy of Chilenje Baby Mix

4.2.3.8 Anthropometric measurements

All anthropometric measurements were carried out by the same two trained assistants who had previous experience in growth monitoring at the Chilenje clinic's Maternal and Child Health department. Measurements of growth (nude weight) and body composition (triceps, biceps, suprailiac and subscapular skinfolds, abdomen, thigh, chest, head and mid-upper arm circumferences) were made in triplicate using standardized anthropometric techniques and calibrated equipment (Lohman et al, 1988) monthly from 6 to 9 months and recorded as shown in Appendix 9-5. Infant's nude weight was mesured to the nearest 100g using a Salter scale.

In order to determine inter-observer variation in measurements each nurse took 3 daily measurements for 3 days of weight, height, cirmferences (chest, abdomen, thigh and mid upper arm) and skinfolds (triceps, biceps, subscapular and suprailiac) of the same 15-year old girl who had volunteered to be measured with

permission from the guardian. There were no significant differences in anthropometric values obtained by the two nurses (Table 4-7). Although we were not able to perform repeated measurements on the same infant to compare anthropometric values obtained by the two nurses, each of them had rehearsal measurements on infants until three measurements agreed within 0.5 units.

Mother's weight was determined to the nearest 100 g using an electronic scale. Infant's recumbent length was measured to the nearest 0.1 cm on a portable measuring board. Triceps, biceps, subscapular and suprailiac skinfolds were measured to the nearest 0.1 mm using Holtain skinfold calipers (Crymych, United Kingdom). Abdomen, thigh, chest, head, and mid-upper arm circumferences were measured to the nearest 0.1 cm using non-stretchable measuring tape.

Table 4-6 Standandization of anthropometric measurements between two trained nurses

Variable	Nurse 1 (n = 9)	Nurse 2 (n = 9)	P
Weight (kg)	46.7 (SD 0.6)	46.8 (SD 0.5)	0.35
Height (cm)	157.7 (SD 0.7)	157.6 (SD 0.5)	0.52
Head	53.2 (SD 0.5)	53.4 (SD 0.1)	0.24
Body circumferences			
Chest (cm)	74.9 (SD 0.5)	74.6 (SD 1.1)	0.31
Abdomen (cm)	69.1 (SD 0.2)	68.9 (SD 0.8)	0.75
Thigh circum (cm)	42.8 (SD 0.3)	43.1 (SD 0.3)	0.30
Mid Upper Arm (cm)	23.0 (SD 0.3)	22.9 (SD 0.3)	0.12
Skinfold thicknesses			
Triceps (mm)	10.2 (SD 1.9)	9.9 (SD 2.1)	0.62
Subscapular (mm)	8.5 (SD 0.2)	8.7 (SD 0.1)	0.06
Biceps (mm)	5.4 (SD 0.8)	5.0 (SD 1.3)	0.32
Suprailiac (mm)	5.2 (SD 0.1)	5.2 (SD 0.3)	0.90

All the anthropometric indicators were measured once in the control infants at 9 months. Weight and length mesurements for infants were converted to Z-scores using EpiNut on Epi Info Software (version 3.2, Centre for Disease Control and Prevention, WHO, 2002). Maternal body mass index was calculated from weight and height using a standard equation (Cole et al, 2000). Body composition data were used to calculate body fat (fat mass) and muscle (fat-free mass), mid arm muscle area (MAMA) and mid arm fat area (MAFA). MAMA and MAFA were calculated using previously reported equations (Lartey et al, 1999).

4.2.3.9 Haemoglobin concentration measurement

Finger prick blood was used to measure haemoglobin concentration twice by HemoCue haemoglobin concentrationometer (HemoCue, Shefield, United Kingdom) in the clinic at 6 and 9 months. Anemia was defined as haemoglobin concentration <110 g/L (WHO, 1968), moderate- to-severe and severe anemia as haemoglobin concentration < 90 g/L and < 70 g/L, respectively (Stoltzfus et al, 1998).

4.2.3.10 Morbidity

Two types of morbidity data (Rollins et al, 2001) namely, 1) overall morbidity in the last month (scored as healthy; mild, self-limited illness; moderate illness requiring symptomatic treatment at the clinic; severe illness requiring antibiotics or other medical intervention); and 2) symptoms (diarrhea, lower respiratory infection, ear suppuration, skin infections, purulent conjunctivitis, oral thrush) within the past 3 days, an interval shown to provide adequate recall data for diarrhea (Boerma et al., 1991) were collected for both infant and mother during their monthly visits to Chilenje clinic using questionnaires shown in Appendix

9-6.

4.2.3.11 Measurement of breast milk intake

Deuterium dose preparation and maternal dosing was done based on a protocol (Appendix 9-7) in use at the Medical Research Council (MRC) Human Nutrition Research, Institute of Child Health, UK. Figure 5-4 shows the deuterium dosing process, insertion of cotton ball into the nappy and transfer of urine sample to microtube.

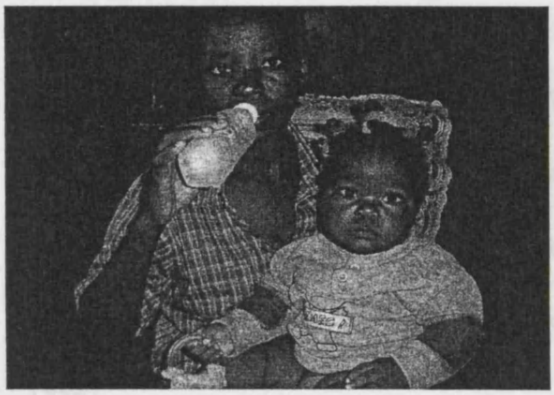
About 10 g of deuterium oxide (^2H_2O) were accurately weighed using an electronic kitchen scale into a 250 ml plastic bottle via 10 ml syringe attached to 0.45 μL micropore filter. 50 ml of filtered tap water were added and bottle was capped before thoroughly shaking the mixture for 1 minute. 1 ml of mixed sample was transferred into a 2 ml micro-tube using 1 ml pipette that was disposed of afterwards. This was marked pre-dose aliquot and was labelled with subject number and date of preparation before storage at -20 °C. The bottle was recapped tightly, put in sealable plastic bag and weighed (WT 1). Samples were stored in a domestic refrigerator overnight for dosing the next day.

The prepared deuterium oxide soultion was taken to the mother's home by a trained nurse. Pre-dose urine samples were obtained from both the mother (into a 30 ml universal sample tube) and infant and the time when urine samples were obtained noted.

To get urine from the infant, cotton wool ball was placed in the nappy and checked every thirty minutes for wetness. The time of the infant's urine sample

was recorded as the average of the time when the cotton ball was noted to be wet and the time it was last checked and found dry in the previous 30 minutes. The wet cotton wool ball was retrieved and put into a syringe barrel. The plunger was inserted and urine pushed into a 2 ml cryo-tube. Maternal weight and height and infant's weight and length were measured at the time of dosing (Day 0). The mother was given the capped bottle containing deuterium sample and was asked to open the bottle and drink the entire sample and the time of dosing noted. After the mother drank the sample, the empty bottle was recapped and placed back in the plastic bag and was weighed later (WT2). The amount of dose given to each mother was calculated as WT1-WT2.

Additional urine samples were collected for the mother on Days 1, 4 and 14 and for the infant on Days 1, 3, 4, 13 and 14. Urine sample tubes were labelled with subject number, date and time of sample collection and stored at -20 ^{o}C. Appendices 9-8 and 9-9 show record sheets for the 2-week urine collection period.



a. Dosing

b. insertion of cotton ball

c. transfer of urine from wet cotton into tube

Figure 4-6 Maternal dosing with deuterium, insertion of cotton ball and transfer of infant's urine sample to microtube

Urine samples and pre-dose aliquots were transported frozen to the United Kingdom for analysis by isotope ratio mass spectroscopy (Davies et al, 1989).

4.2.3.11.1 Sample isotope ratio definition

Sample ratio mass is in practice not reported in absolute terms, but as the deviation (delta) of the isotope abundance ratio from the abundance ratio of the international reference material known as Vienna Standard Mean Ocean Water (VSMOW) (van Trigt et al, 2001). The isotopic abundance of a sample is expressed in parts per thousand (‰) difference from the standard (Coplen, 1994). The conventional value of VSMOW is 0‰ (part per thousand) (Izbicki et al, 1998).

Isotopic abundance is calculated according to equation 4-2 modified from McKenchie et al (2004).

$$\delta = \left[\frac{R_{Dsample} - R_{DVSMOW}}{R_{DVSMOW}} \right] * 1000 \qquad \text{..... Equation 4-2}$$

where δ is sample isotopic abundance (‰)
$R_{Dsample}$ is the ratio of deuterium (^2H) to hydrogen (^1H) in the sample
R_{Dvsmow} is the ratio of deuterium (^2H) to hydrogen (^1H) in the VSMOW

4.2.3.11.2 Calculation of breast milk intake

Breast milk intake was calculated as described by workers from Brazil (Haisma et al, 2003). Briefly, isotopic data were fitted to a model for water turnover in the mother and infant transfer of milk from mother to the infant using equations 4-3 and 4-4 for mother's and infant's data, respectively.

$$E_{m(t)} = E_{m(0)} e^{-\frac{F_{bb}}{V_b} \cdot t} \qquad \text{..... Equation 4-3}$$

where $E_{m(t)}$ is isotopic enrichment above background at time t (ppm), $E_{m(0)}$ is the zero time isotope enrichment (ppm), t is time post-dose (d) and K_{mm} is water turnover in the mother (1/d).

$$E_{b(t)} = E_{m(0)}\left(\frac{F_{bm}}{V_b}\right)\left[\frac{e^{-K_{mm}\cdot t} - e^{-\frac{F_{bb}}{V_b}\cdot t}}{\frac{F_{bb}}{V_b} - K_{mm}}\right] \quad \text{..... Equation 4-4}$$

where $E_{b(t)}$ is isotopic enrichment above background at time t (ppm), F_{bm} is the transfer of water from the mother to the infant via breast milk (kg/d). $E_{m(0)}$, F_{bm}, K_{mm} *and* F_{bb} were fitted using the Microsoft Excel® "Solver" function in order to minimize the sums of the squares of the differences between observed and fitted values for combined data for mother and infant as shown in Appendices 9-10.

V_b is the infant's total deuterium distribution space (kg) and F_{bb} is total water loss in the infant (kg/d). V_b was assumed to vary linearly with weight (kg) as depicted in equation 4-5.

$$V_b = 0.84W^{0.82} \quad \text{..... Equation 4-5}$$

Breast milk volume was extrapolated from infant's isotopic dilution space based on the following equations and assumptions (Haisma et al, 2003).

Equation 4-6: Breast milk intake, $M = F_{bm}/0.871$, (breast milk is 87.1% water)

Equation 4-7: Total water input from breast milk, $F_m = F_{bm} + 0.09M$,

(water in breast milk and the water of oxidation of breast milk solids add to about 9g water/100g breast milk)

Equation 4-8: Water gained in growth during 14 experimental days,

$F_g = (V_{b,14d}-V_{b,0d})/14$

Equation 4-9: Infant's total water output, $F_{ob} = F_{bb}/0.9915$,

(correction for isotopic fractionation assuming that 15% of the infant's water losses were fractionated).

Equation 4-10: Non-oral water intake, $F_a = 0.063M$,

(water exchange between alveolar and the atmosphere estimated as 6.3% of milk intake).

Equation 4-11: Non-milk oral water intake, $F_s = F_{ob}- F_m- F_a + F_g$

Daily nutrient intake from breast milk was calculated from the daily breast milk intake based on the nutrient composition of mature breast milk presented in Table 4-8.

Some mothers (n = 10) and infants (n = 11) had positive pre-dose enrichments. This unexpected result may be due to the presence of deuterium in in drinking water. The overall average for mothers and infants with ne.g.ative pre-dose enrichment values were applied in curve fitting for cases with positive pre-dose values. The average pre-dose values were -15.5 (SD 7.4; 95% CI: -18.7, -12.2) and -13.4 (SD 6.5; 95% CI: -16.8, -10.0)‰ VSMOW (Vienna Standard Mean Ocean Water) for mothers and infants, respectively. Ne.g.ative sample

enerichments are obtained when sample isotopic enrichment is lower than that of the VSMOW. The isotopic enrichment of the tap water (Institute of Child Health, London) was -42.0 (SD 3.1)‰ VSMOW. This value is higher than the enrichment reported for tap water (-61.2)‰ VSMOW) in Sweden (Gudmundsson, 2001). The difference may be explained by evaporation and isotopic fractionation (Abruzzese et al, 2005)

Infants were grouped according to breast milk intake level (low breast milk defined as intake < 450 g/d, medium as 450 – 750 g/d and high as 750 g/d and above) in order to assess the effect of this on complementary feeding pattern. 450 g/d was chosen since it was the median intake for all the three groups. The cut-offs for low breast milk intake used in this study were based on a global average breast milk intake of 565 g/d (Dewey and Brown, 2003) for infant and young children 6 – 24 months old . Appendices 9-11 and 9-12 depict curve fitting for infants with low (mother and infant curves do not touch at all or touch later) and high (mother and infant curves touch earlier) breast milk intake, respectively.

Table 4-7 Nutrient composition of mature breast milk

Nutrient	Amount	Reference
Energy (kcal/100g)	67	Dewey and Brown, 2003
Protein (g/100g)	1.4	Goes et al, 2002; Hosoi et al, 2005
Fat (g/100g)	1.8	Goes et al, 2002
Calcium (mg/L)[1]	210	Abrams and Atkinson, 2003
Iron (mg/L)	0.35	Lynch and Stoltzfus, 2003
Zinc (mg/L)	1.5	Goes et al, 2002
Copper (mg/L)	0.48	Goes et al, 2002
Vitamin C (mg/L)[2]	47	Daneel-Otterbech et al, 2005
Vitamin A (μg RE/L)	500	Mora, 2003
B vitamins		Allen, 2003
Thiamine (mg/L)	0.21	
Riboflavin (mg/L)	0.35	
Niacin (mg/L)	1.8	
Pyridoxine (mg/L)	0.13	
Folic acid (μg/L)	85	
Vitamin B-12 (μg/L)	0.42	
Pantothenic acid (mg/L)	2.2	
Biotin (μg/L)	8	
Choline (mg/L)	160	

[1]for infants aged 7-12 months
[2] average of the breast ascorbic acid content for African women (31 mg/kg) and European women (60 mg/kg)

4.2.3.12 Dietary intake measurement

Dietary data were collected using interactive 24-h recall (Appendix 9-13) as detailed previously (Gibson and Ferguson, 1999). A single 24-h recall session was performed monthly for three consecutive months. A total of 65 recipes were identified and used to create a food composition table (Appendix 9-14) on as-is-eaten basis. Ten food groups were generated from the identified recipes namely, 1) cereals and cereal products, 2) porridge with groundnuts, 3) Chilenje Baby Mix, 4) milk and milk products, 5) commercial complementary foods (milk and soya-based), 6) e.g.gs, 7) soups, 8) fruits, 9) beverages (fruit juices, soft drinks), 10) others.

Dietary data were used to calculate: 1) age-specific amount of Chilenje Baby Mix and other food groups consumed in grams and kilocalories; 2) proportion of the total daily energy and dietary intake contributed by the Chilenje Baby Mix and the other food groups. To calculate individual nutrient intake SPSS syntax (Appendix 9-15) created from the food composition table described above (see Appendix 10-18) was run over SPSS database (Appendix 9-16) showing the grams of each food eaten (from 24-h recall sheets), subject ID, subject group (1 = CBM, 2 = CBMA or 3 = Control), food name, food code and time eaten (B = breakfast, L = lunch, S = Snack, D = Dinner, A = all day).

4.2.3.13 Data analysis

Qualitative findings were analysed by VO based on previous guidelines (Creswell, 1994; Kitzinger, 1995). Tape records of the three focus group discussions were transcribed verbatim and combined with a review of notes that were taken by VO and an assistant. The transcripts were coded into major themes that were generated manually. Internal validity was ensured by combining focus group discussions with interview and home observation data. Since findings from all three techniques were similar, results were combined. Secondly, peer examination of the transcripts was done by an African nurse with an MSc and experience in nutrition who was not directly connected with the study. Quantitative data from home observation were double entered using Epi Info (version 3.2, Centre for Disease Control and Prevention, WHO, 2002). Frequencies, means and median values were calculated using SPSS software (version 11.6).

Data from the randomized controlled trial were double entered using Epi Info (version 3.2, Centre for Disease Control and Prevention, World Health Organisation, 2002). Stunting, underweight and wasting were defined as length-for-age, weight-for-age and weight-for-length, respectively, < -2 standard deviations of the NCHS/WHO reference standards (WHO, 1983). Moderate-to-severe and severe malnutrition in infants were defined as MUAC < 12.5 cm and MUAC < 11 cm, respectively (Cogill, 2003).

Frequencies, means and median values were determined using the Statistical Package for Social Scientists software version 11.0 (Chicago, Illinois, USA). Differences in non-continuous variables between interventions and between intervention and control were determined by Kruskal Wallis test for K independent samples. Differences in continuous variables between intervention and control groups were tested by one way analysis of variance (ANOVA) and between groups by post-hoc multiple comparisons (least square difference). Differences in continuous variables between CBM and CBMA were determined by independent samples t-test. It is noted that since there were no significant differences between CBM and CBMA in growth, haemoglobin concentration and dietary intake, most of the results of the randomised trial are presented giving a combined ANOVA comparison between the two interventions (CBM and CBMA) versus the control group. However, in cases like length where only one intervention group (CBM) was significantly different from the control group, p values are given for both combined intervention versus control and multiple comparison p value for comparison between that intervention group and the control group. Since length was not measured in the control group at 6 months, no

length gain data comparison is presented. Protein Efficiency Ration (PER) and Net protein utilisation (NPU) for CBM and CBMA were calculated using previous equations (Bender, 1956):

PER = gain in weight (6-9 moths) g/g protein (6-9 months)..... Equation 4-12

NPU = 40 + 12.6*PER ..... Equation 4-13

PER and NPU values for CBM and CBM were used to estimate PER and NPU values for the preliminary recipes developed in the second phase of the study based on blend protein content.

4.2.3.14 Ethical approval

Ethical approval was obtained from the University of Zambia Ethics Committee and Great Ormond Street Hospital GOSH) and Institute of Child Health (ICH) Ethics Committee, UK. Mothers and other study participants gave written informed consent.

5 Results

This section is divided into three sub-sections namely, 1) complementary feeding practices, 2) development and acceptability of study complementary food and, 3) randomised controlled trial.

5.1 Complementary feeding practices

5.1.1 Focus Group Discussion Themes

Five major focus group discussion themes (Boxes 5-1, 5-2 and 5-3) were generated: 1) breastfeeding and timing of weaning and 2) food availability and affordability; 3) food beliefs and attitudes and 4) food choice and avoidance; and 5) food preparation.

5.1.1.1 Breastfeeding and timing of weaning

There was wide knowledge of the benefits of breastfeeding for the first two years of life. The need to feed on demand was also supported, especially for younger infants and in cases where mothers were not working outside the home. Even though there was understanding of the benefit of breastfeeding, there were doubts as to the nutritional adequacy of breast milk. Complementary foods were introduced mostly when the child attained 4 – 6 months, however, the age of introducing these foods ranged from 1 – 9 months. Factors determining the timing of complementary foods included baby crying, and baby not being satisfied. The other factors were advice from the health clinics and the necessity for some

mothers to return to work. Knowledge of maternal HIV status was also mentioned as one of the factors that may lead to early introduction of complementary foods.

5.1.1.2 Food availability and affordability

The main foods available for feeding infants included maize meal, rice, groundnuts, beans, fish, milk and fruits (bananas, oranges, pawpaw) and ve.g.etables (carrots, pumpkins, green ve.g.etables). The child's food was determined by what was available in the household, the cost of food and seasonality. Even though commercially processed complementary foods were also available in the market, their use for feeding infants was restricted by the high prices.

Box 5-1 Focus Group Discussions (themes 1 and 2)

Breastfeeding and timing of weaning

Quotes

"...normally six months they introduce [complementary foods], *but in between you breastfeed, like five times a day''* (father).

"...it is our duty to breastfeed on demand'' (mother).

"..any time when the baby wants to breastfeed for those who are not working'' (nurse).

"...yes it is understood it [breast milk] *has all the nutrients, but to supplement to satisfaction of the baby you need more food, the baby needs solids"* [father].

"... when baby cries a lot, they can start at one month because child is hungry'' (mother).

"... sometimes it is at the clinic, where they tell mothers the right thing, like me it was the Sister" (mother).

"...when mothers go to the clinic, from knowledge from clinic, babies should introduce foods after six months'' (father).

"... the caretakers, the mother is at work, so the caretaker decides to give [solid foods]" (nurse).

"... these days it is the [mother's HIV] *status that matters"* (mother).

Food availability and affordability

Quotes

"...whatever is available in the family, even biscuits'' (nurse).

"...groundnuts, it depends on the season, like now [September] *it is expensive"* (mother).

"I have never used them [commercial complementary foods] *because I cannot afford... they are not very common to all, may be a few families, ...those who can afford them use them, if they are available they are used, they are there in the shop, people are buying them, but not us"* (nurse).

5.1.1.3 Food beliefs and attitudes

There was preference for foods prepared at home as mothers felt that they were fresher and that they contained all the nutrients. However, there was also acknowledgement that some of the foods prepared at home may cause stomach problems to the child. The use of traditional foods as opposed to commercially prepared foods was thought to be more common among poorer households. There

was acknowledgement that commercially processed complementary foods were easy to prepare and some participants felt they were better than what they prepared at home. However, the use of these foods was hampered by mistrust of their freshness and nutritional completeness and unaffordable prices.

5.1.1.4 Food choice and avoidance

Food choice was affected mainly by the food's nutritional value, availability, cost and the child's age. Other factors included expiry date and temperature. There was emphasis among focus group participants on the provision of a varied diet. With additional income, households buy foods they consider to be more nutritious. Foods were considered bad if they were carbonated, spiced, acidic or too sweet.

5.1.1.5 Food preparation

The main food for infants was porridge that was prepared in different ways depending on ingredient availability. Cooking of porridge lasted 20 – 45 minutes, but whether porridge is well cooked is measured by some indicators. The other major food for infants is *nshima* (a stiff porridge from maize meal) and is prepared such that the child can easily swallow. Mother's availability had a major effect on what food the infants were fed and how these foods were prepared and meal frequency. In most cases the child was left in the care of a househelp or siblings who may not fully understand the child's feeding needs.

Box 5-2 Focus Group Discussions (themes 3 and 4)

Food beliefs and attitudes

Quotes

"... some think with maize meal porridge their children may suffer from stomach problems, so they give Cerelac and Vitaso" [commercial infant foods] (mother).

"... rich people feed with expensive foods, they think groundnuts are for poor people, people will laugh" (mother).

"I have never believed in them [commercial complementary foods] *simply because I have never witnessed some being prepared, secondly, I never know how long they have been standing in the shelf and since I believe in food prepared at home, that is why I have never taken my time to go out and give my children things from the shop"* (nurse).

"... you cannot be sure they put everything they indicate on the boxes" (mother).

"I think some people think those which are already made they are not very good because even the ingredients, they just dry it, they are not there, they are not even meant for babies, they just put baby there and say these are for baby" (father).

Theme 4: Food choice and avoidance

Quotes

"... any food as long as it has the vitamins required for her" (mother).

"... like what I have done with my daughter, I always use a bit of milk with sugar, and if I have money I add some soya" (father).

Box 5-3 Focus Group Discussions (theme 5)

Food Preparation

Quotes

"... mostly when we use maize meal it goes according to homes, some they add sugar, others add pounded groundnuts, some add salt, ... sometimes they go to an extent of saying in case there is no sugar then you put salt or just like that, but mostly we put sugar" (father).

"... most of the time I give her maize meal with groundnuts. I measure water with a small cup. I put two cups, then I add some groundnuts, one tablespoon and two tablespoon, and a half maize meal. I add two tea spoons of sugar and a pinch of salt. I stir it. For me to know that this porridge is ready, one cup must go, one cup must remain" (mother).

"... for nshima, you put pot of water on fire, wait for it to be hot, then you make like porridge, wait for it to boil, start adding mealie meal bit by bit while you continue stirring, until you see it starts becoming very thick, but not very strong, when it is ready then you put the lid on top of the pot and wait, that is when you can put it on a plate. I think even for the baby it is like that but you should not make it very hard so that it is easy for the baby to swallow" (father).

"I think these days when you look at these mothers most of them they are lazy in cooking porridge for the babies, so you find most of the time they just add groundnuts with milk, this addition of fish, kapenta [small dried fish], *beans, they rarely use them because they have got no time"* (nurse).

5.1.2 Interviews

Table 5-1 presents breastfeeding and complementary feeding practices of interviewed mothers.

Table 5-1 Breastfeeding and complementary feeding practices determined by interviewing 34 mothers

Mothers currently breastfeeding	88%
Frequency of breastfeeding	
Day	7 (SD 4)
Night	5 (SD 3)
Child's age (months) when mother plans to stop breastfeeding	19 (SD 4)
Percent mothers feeding other milks	53%
Feeding fresh cow's milk	44%
Feeding Lactogen™	6%
Other tinned powdered milk	3%
Infant's age (months) at which complementary foods are introduced	6 (SD 2)
Reason for introducing complementary foods at particular age[1]	
Child crying	32%
Advice from health worker	23%
Lack of enough breast milk	6%
Advice from relatives	6%
Child old enough	6%
Mother working	9%
Most important factor considered in food choice for infants[1]	
Nutritional value	53%
Freshness	26%
Food temperature	23%
Child's age	12%
Child's food preference	12%
Daily cooking frequency (infant's food)	3
Range of porridge cooking duration (minutes)	30 – 60

[1]mothers were allowed to give more than one response

5.1.2.1 Use of le.g.umes, cereals and pre-packed complementary foods and food preparation

For preparation of infant foods, all mothers used maize, beans and groundnuts, while a third of mothers used bambaranuts and pre-cooked soya flour. Maize-meal-based porridge was the main form of food for infants. Porridge was prepared with the addition of any or more of the following ingredients to maize meal: pounded groundnuts, cooking oil, sugar, fresh milk, salt, e.g.gs and custard powder.

The main pre-packed multi-micronutrient fortified cereal-based complementary foods available in Lusaka were high energy protein supplement (HEPS, a maize-soya food mainly used as food aid in relief operations, but also available in a few shops), Speciality Vitaso™, Speciality Nutrex™ and Cerelac™. These foods are mainly targeted at children from 6 months to 4 years of age.

The average price per kilogram for the most commonly used commercial complementary foods in Lusaka was found to be about US$ 4.0. Table 5-2 shows commercial complementary foods purchased from shops by mothers and their prices.

Most mothers in Chilenje cannot afford the commercial complementary foods. Soy bean-based complementary foods have been produced in Zambia since 1950s (Royal Tropical Institute, 1983), however, according to key informants in this study, the distribution of these foods has been driven more by donor agency demand than commercial sustainability. In addition, soy-bean production in Zambia is under large-scale holding. Further, due to the hard-to-cook nature

soybeans attributable to hard seed coat, soy beans may not be easily processed using house-hold level techniques.

Table 5-2 Commercial complementary foods purchased from shops by mothers and their prices

Food name	Target group	Ingredients	Multi-micronutrient fortified	Price/kg (US$)
HEPS[1]	General population	Soya, skimmed milk powder, maize, sugar	Yes[2]	2.50
Vitaso	6 months – 4 years	Soya, maize, sugar	Yes[2]	3.1
Nutrex	6 months – 4 years	Soya, skimmed milk powder, maize, sugar	Yes[2]	3.1
Cerelac	≥ 6 months	Wheat, skim milk powder	Yes	8
Lactogen	≥ 6 months	Milk powder	Yes	8
Pronutro[3]	≥ 6 months	Soya, skimmed milk powder, maize, sugar	Yes	8
Purity	≥ 4 months	Cereal with fruit essence	Yes	4

[1]High Energy Protein supplement, mainly distributed by relief agencies.
[2] products, especially HEPS ortified at levels targeting wide age range and may not supply adequate micronutrient amounts to infants.
[3] was rarely used

5.1.2.2 Cash expenditure on food and fuel

The mother's ability to purchase food for their child was determined mainly by educational attainment, which may have affected salary level, and marital status. Mothers who had attained at least primary, secondary and tertiary level of education made spending decisions in 25%, 32% and 83% of the cases, respectively. While married mothers made spending decision in only 27% of cases, single mothers decided in 75% of cases. 82% of mothers used electric hot plates for cooking, while 18% used charcoal. The mean monthly electricity cost

was US$ 12. Lack of electricity occurred mostly twice a week, but this became worse during rains and 59% of mothers recalled having experienced electricity failure in the month prior to the interview.

5.1.3 Home observations

5.1.3.1 Dietary intake

Table 5-3 presents the daily intake of energy from traditional complementary foods measured by 12-h weighed food records and 24-h recall and from breast milk for infants 6-8 months, 9-11 months and young children aged 12 – 18 months old (Dewey and Brown, 2003). Table 5-4 shows the daily intake of thiamine, riboflavin, niacin, vitamin A, iron, calcium and zinc from complementary foods calculated from 12-h weighed food record and 24-h recall and from breast milk for infants and young children 6-18 months old (Dewey and Brown, 2003). Although the two techniques were used to determine food intakes for two different days, the results were similar with both techniques. The mean energy density (based on recipes from 12-h food record and 24-h recall) of home porridges was 3.8 (SD 1.1) kJ/ml and 3.5 (SD 1.1) kJ/ml, respectively for recipes obtained by 12-h weighed food record and 24-h recall. However, recipes obtained by 24 h had higher absorbable iron density compared to those obtained by 12 h weighed food record. This may be attributed to inability of mothers to remember the actual amounts of porridge ingredients during 24-h recall session.

The overall average daily food solids intake from complementary foods was 127 g (95% CI, 82.7, 171.3) for the three age groups [82 g, 146g and 153 g for infants 6-

8, 9-11 and young children 12-18 months, respectively. The average daily intake of protein and fat were 10.6 g (95% CI, 5.2, 16.0) and 11.6 g (95% CI, 6.3, 16.8), respectively, representing 107% and 179% of RDA for protein and fat, respectively for children aged 6 – 18 months.

5.1.3.2 Infant feeding and care

In most cases (70%) the mother fed the child. The rest of the time the child was fed by the grandmother, sibling or house-help. The feeding location was predominantly (75%) in the sitting area of the family house with the child held on mother/caretaker's lap. Verbal encouragement was observed in 74% of the cases, and in most cases was occasioned by the child's refusal to eat. Predominant silence with occasional encouragement or coaxing was observed in 26% of the cases. It is noteworthy that the presence of observers may have interfered with normal feeding habits. Feeding utensils comprised plastic cups and spoons for porridge. Other foods were served on plastic plates and fed by hand. Most mothers (68%) did not store leftover food, which in most cases was eaten by an older member of the family (mother, sibling or any other member of the household).

Table 5-3 Daily energy intake (MJ/day) from traditional complementary foods calculated from 12-h weighed food record and 24-h recall and from breast milk for infants 6-8, 9-11 months and young children 12-18 months old.

	Age range					
	6-8 months		9-11 months		12-18 months	
	12-h weighed food record	24-h recall	12-h weighed food record	24-h recall	12-h weighed food record	24-h recall
Energy intake (MJ/day)						
Complementary foods energy [mean (95% CI)]	1.2 (0.6; 1.7)	1.4 (0.9; 1.9)	2.7 (0.9; 3.2)	2.0 (1.6; 2.5)	2.9 (0.9; 4.3)	2.0 (1.0; 3.0)
Breast milk energy[1]	1.7		1.6		1.4	
Percent RDA[2]	112	119	151	127	115	91

[1]Values are for average breast milk intake taken from Dewey and Brown, 2003

[2]Recommended Dietary Allowance (RDA) is used throughout this thesis to compare infant nutrient intakes to requirements to allow for comparison of this study with other studies on complementary feeding of infants. Recommended Nutrient Intake (RNI) derived by factorial method is the United Kingdom equivalent of RDA. RDAs for infants' calcium and vitamin A intakes are lower than RNI for the two micronutrients (Lutter and Dewey, 2003). RDAs for B-vitamins are similar to the RNIs except for folate recommendation that is lower with RNI (Lutter and Dewey, 2003).

Table 5-4 Daily intake of thiamine, riboflavin, niacin, vitamin A, iron, calcium and zinc from complementary foods calculated from 12-h weighed food record and 24-h recall breast milk for infants and young children 6-18 months old.

	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin A (μg)	Iron[2] (mg)	Calcium (mg)	Zinc (mg)
Intake Complementary foods [mean (95% CI)]							
12-h record	0.3 (0.1, 0.4)	0.2 (0.1, 0.6)	2.7 (1.1, 4.9)	185 (73, 341)	0.2 (0.06, 0.3)	44 (9.7, 65)	2.2 (1.7, 3.4)
24-h recall	0.4 (0.2, 0.6)	0.3 (0.2, 0.5)	3.4 (1.4, 5.3)	165 (72, 258)	0.2 (0.08, 0.2)	121 (29, 214)	2.0 (1.5, 2.8)
Breast milk[1]	0.13	0.13	1.1	307	0.1	130	0.3
Percent RDA[3]							
12-h record	124	78	88	131	55	34	61
24-h recall	161	109	79	129	54	54	56

[1]Values are for average breast milk intake taken from Dewey and Brown, 2003

[2]Average iron absorption from non-breast milk foods assumed to be 4%. Range of iron absorption for maize-based meals is 2.4-6%. Results were calculated by dividing total daily iron intake by absorbable iron RDA: 0.58 mg /day and 0.54 mg /day for infants 7-12 months old and children 13-24 mo old (Lynch and Stoltzfus, 2003).

[3]Recommended Dietary Allowance (RDA) is used throughout this thesis to compare infant nutrient intakes to requirements to allow for comparison of this study with other studies on complementary feeding of infants. Recommended Nutrient Intake (RNI) derived by factorial method is the United Kingdom equivalent of RDA. RDAs for infants' calcium and vitamin A intakes are lower than RNI for the two micronutrients (Lutter and Dewey, 2003). RDAs for B-vitamins are similar to the RNIs except for folate recommendation that is lower with RNI (Lutter and Dewey, 2003).

5.2 Development, acceptability and costing of complementary food

5.2.1 Nutritional properties of processed maize-beans-groundnuts- bambaranuts blends.

Table 5-5 shows the calculated nutritional composition and estimated basic cost (US$/kg) of the three maize-bean-bambaranut-groundnut (65:15:5:15; 65:15:10:10 and 70:10:10:10) recipes used for preliminary acceptability assessment. All the three recipes closely met the requirements for protein and fat energy (Lutter and Dewey, 2003) composition for complementary foods for infants and young children.

Table 5-5 Estimated energy composition, Protein Efficiency Ration (PER), net protein utilization (NPU) and basic price of three preliminary recipes compared with reference values.

	Recipe (Ingredient ratio)[1]			
	65:15:5:15	65:15:10:10	70:10:10:10	Reference value[2]
Protein (g/100kcal)	3.6	3.6	3.5	≥ 2.5
Fat (g/100kcal)	2.5	2.0	2.1	$\geq 2, \leq 6$
% protein energy	14.30	14.53	13.80	6 -10
% CHO energy	63.1	67.2	67.6	62-70
% fat energy	22.6	18.3	18.6	24-28
PER	3.0	3.0	2.9	
NPU[3]	78	78	75	
Price(USD) per Kg	0.59	0.59	0.58	NA

[1]maize:beans:bambaranuts:groundnuts (%w/w) ratio;
[2] estimated needs of children 6-24 months taken from Lutter and Dewey, 2003.
[3]The NPU for CBM and CBMA observed in the current study are higher than the estimated average value, 69.1 (SD 12) for 12 West African complementary foods containing groundnuts, cereals (maize, millet or sorghum) and another legume (Onofiok and Nnanyelugo, 1998).

5.2.2 Acceptance of blends by mothers

All of the roasted porridges were generally well accepted by mothers with most giving the different attributes scores of 1 or 2. Table 5-6 shows the Wilcoxon signed rank test for effect of α-amylase treatment and Friedman's significance for the effect of porridge recipe on roasted maize-bean blend acceptability by mothers. There were no significant differences in colour, smell, thickness, taste or overall acceptability among the three porridge recipes. However, there was a tendency towards more liking of recipe 1 and α-amylase treated porridges. Post-tasting comments by mothers concerning the α-amylase-treated blends included: *"baby will like"*; *"smell and taste are stronger, therefore tastier"*; *"right thickness"*; *"good for babies 2-10 months* [old]*"*; *"had a bit of sweetness"*; "*nice without sugar"*. Mothers' comments on the non-α-amylase-treated porridges were: "*too thick"*; *"plain"*; *"taste bad"*; *"tasteless"*. It is noted that these comments on non- α-amylase-treated porridge were all related to thickness and taste, both of which are modified with the addition of the enzyme. Mothers observed that all the porridges under test were better than those they prepared at home due to the nutty taste.

5.2.3 Effect of α-amylase on infant porridge intake

Mothers were given coded samples of roasted porridges to take home and prepare on different days. Mothers were asked to record how much porridge was served and how much was left after feeding using graduated feeding cups that were provided. A comparison of porridge intake showed that infants consumed 99 (SD

41) ml, 109 (SD 53) and 123 (SD 48) ml of porridge representing 64%, 69% and 80% of servings of traditional porridges, α-amylase-treated maize-bean porridge, and non-α-amylase-treated maize-bean porridge, respectively. The observation that infants consumed more of the non-α-amylase-treated maize-beans-bambaranuts-groundnuts blend compared to α-amylase treated porridge is consistent with previous observations that children consumed more low-energy dense food than high-energy dense food (den Besten et al, 1998; Vieu et al, 2001). It is noted that the method employed in the current study was semi-quantitative as amounts of maize-beans-bambaranuts-groundnuts porridge as reported by mothers were compared with amounts of traditional porridge determined by weighed food records.

Table 5-6 Effects of α-amylase treatment and Friedman's significance for the effect of porridge recipe on maize-bean-bambaranut-groundnut blend acceptability by mothers[1].

Recipe[2]	Preference	Colour	Smell	Taste	Thickness	Overall preference
1	non-α-amylase	3	7	2	2	3
	α-amylase	4	5	5	5	8
	tie	11	6	11	11	7
2	non-α-amylase	4	4	4	3	2
	α-amylase	2	3	5	6	4
	tie	12	11	9	9	12
3	non-α-amylase	3	5	5	2	3
	α-amylase	3	5	4	4	5
	tie	12	8	9	12	10
Order of decreasing preference[3]						
non-α-amylase porridges		1,3,2	2,1,3	3,2,1	3,2,1	3,2,1
α-amylase porridges		1,3,2	NA	NA	2,3,1	1,3,2

[1]Numbers in the table rows for each recipe indicate the number of mothers with a given preference for amylase or non-amylase treated porridge; there were significant differences by Wilcoxon sign rank test;
[2] Maize-beans-bambaranuts-groundnuts ratio (1 = 65:15:5:5; 2 = 65:15:10:10; 3 = 70:10:10:10)
[3]The order of preference for each porridge recipe was analyzed by Friedman's asymptotic test; there were no significant differences (NA implies recipes had similar rank).

5.2.4 Acceptability and nutritional composition of recipe (65:15:5:15) adopted for study

Recipe 65:15:5:15 which had been most accepted by mothers as described above was further processed by extrusion cooking and by adding α-amylase and fortifying with micronutrients. The blends were liked by mothers because of the taste and shorter cooking times compared to the home porridges. However, most mothers mentioned that they did not like the porridge colour which turned greyish after cooking. The grey colour was mainly due to the dark colour of the variety of bean (*kabulangeti*) used for blend processing. However, other factors including micronutrient premix colour and chemical reactions during extrusion cooking may have affected porridge colour and are discussed in more detail in section 6. Table 5-7 presents the nutritional composition per 100g of roasted and extrusion cooked maize-beans-bambaranuts-groundnuts blend (65:15:5:15).

Table 5-7 Nutritional composition per 100 g by processing method of the maize:beans:bambaranuts:groundnuts recipe (65:15:5:15) adopted for study.

Variable	Blend processing method	
	Roasting	Extrusion cooking
Moisture (g)	8.0 (SD 0.1)	6.0 (SD 0.3)
Protein (g)	15.0 (SD 0.1)	16.0 (SD 0.3)
Fat (g)	11.0 (SD 0.3)	12.4 (SD 0.6)
Crude fibre(g)	4.0 (SD 0.4)	3.4 (SD 0.5)
Carbohydrates (g)	60.0 (SD 0.6)	60.0 (SD 1.0)
Total ash (g)	2.0 (SD 0.1)	2.6 (SD 0.2)
Energy (kcal)	399.0 (SD 1.1)	415.0 (SD 3.0)
Percent energy as protein	15.0 (SD 0.7)	16 (SD 0.5)
Percent energy as fat	25.0 (SD 0.1)	30 (SD 0.2)
Percent energy as carbohydrates	60.0 (SD 0.6)	58 (SD 0.5)

5.2.5 Effect of α-amylase on porridge viscosity and slurry concentration

Application of α-amylase at 0.04% w/w enhanced porridge acceptability and resulted in 88% and 122% increase in flour concentration for roasted and extrusion-cooked porridge flour, respectively, while maintaining porridge viscosity at 1000-fold lower than the viscosity of traditionally used porridges. Traditional porridge viscosities were 2.3 x 10^6 (SD 5 x 10^4) cp, 1.5 x 10^6 (SD 5 x 10^4) cp and 1.8 x 10^5 (7 x 10^3) cp, respectively for maize meal-groundnut, maize meal-cooking oil and maize meal-soya-milk based porridges. The energy densities for α-amylase-treated roasted and α-amylase-treated-extrusion-cooked blends were 68 kcal/100 ml and 80 kcal/100 ml, respectively at 17% and 20% slurry concentration (Figure 5-1). Three servings each of 200 ml can provide the

entire current requirement for energy from complementary foods for infants and young children 6-23 months old.

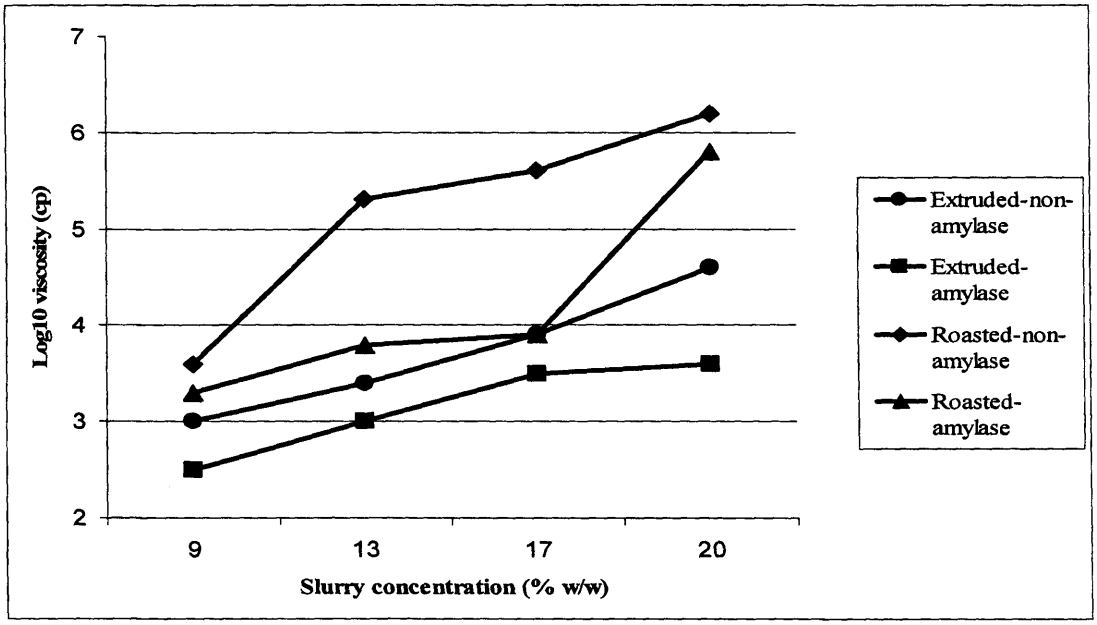


Figure 5-1 Effect of treatment of flour with α-amylase (0.04% w/w) on porridge slurry concentration[1].

[1]Starch hydrolysis during extrusion cooking (Moraru and Kokini, 2003) as a result of high shear stress may explain the observation that extruded blends had lower viscosity than roasted blends.

5.2.6 Microbiological, chemical and sensorial stability of the complementary blend at 25 °C

Table 5-8 shows moisture content, water activity (a_w), peroxide value (meq/kg), yeasts and moulds (cfu/g), and total plate count (cfu/g) and sensory scores (aroma, texture and appearance) for blends that had been sorted for 2 weeks and 6 months at ambient temperature (25 °C). The moisture content (5.8%) and water activity (0.5) observed for the study blend were lower than the optimum requirements for mould growth and aflatoxin production (Moss, 1991). Moulds generally grow at

moisture content above 12% and water activity above 0.7 (Moss, 1991). The optimum water activity for aflatoxin production is about 0.95 (Faraj et al, 1991) at 30 °C (Ogunderu, 1987). The peroxide values observed were below the maximum limit of 10 meq/kg (Gopaldas et al, 1988).

Although the 2 week-old flours had 10-fold higher microbial count, which may have been due to sampling, the overall microbiological load for all blends were within the specified maximum level of 50000 cfu/g for cereal foods for infants and young children (Codex Alimentarius, 1981). Six-month old blends had significantly higher acceptance (lower score on scale) for aroma, while the 2-week old blends had significantly greater acceptance for texture and appearance.

Nevertheless, all of the six blend samples had scores below value 5 showing overall liking by panelists. These results, especially for aroma agree with the low peroxide values observed, indicative of no rancid flavour.

Table 5-8 Moisture, water activity, peroxide value, yeasts and moulds and total plate count and sensory attribute scores for samples of complementary blend stored at ambient temeperature for 2 weeks and 6 months after processing[1,2,3,4].

	Moisture content (g/100g)	Water activity	Peroxide value (meq/kg)	Yeasts and moulds (cfu/g)	Total plate count (cfu/g)	Aroma	Texture	Appearance
Sample								
1	5.7 (SD 0.07)	0.48 (SD 0.02)	1.2 (SD 0.02)	250	3.6×10^3	3.3 (SD 1.4)[a]	4.6 (SD 1.9)[c]	4.2 (SD 1.7)[e]
2	6.8 (SD 0.23)	0.53 (SD 0.01)	0.6 (SD 0.01)	33	2.5×10^4	4.7 (SD 1.8)[b]	2.4 (SD 0.8)[d]	2.8 (SD 1.3)[f]
3	6.2 (SD 0.11)	0.51 (SD 0.01)	1.2 (SD 0.02)	45	2.1×10^3	3.8 (SD 2.0)[a]	4.7 (SD 1.8)[c]	4.3 (SD 1.8)[e]
4	5.9 (SD 0.23)	0.52 (SD 0.01)	1.4 (SD 0.01)	46	2.8×10^4	4.9 (SD 2.1)[b]	2.4 (SD 0.7)[d]	3.3 (SD 1.8)[f]
5	5.8 (SD 0.1)	0.52 (SD 0.01)	0.6 (SD 0.00)	220	2.0×10^3	3.5 (SD 1.7)[a]	4.6 (SD 1.6)[c]	4.3 (SD 1.3)[e]
6	5.8 (SD 0.23)	0.52 (SD 0.01)	0.8 (SD 0.01)	340	5.0×10^4	4.7 (SD 1.7)[b]	2.5 (SD 0.9)[c]	2.9 (SD 1.6)[f]

[1]Six blend samples were defined as: 1) 6-month old non-amylase-treated-non-fortified, 2) 2-week old non-amylase-treated-non-fortified, 3) 6-month old amylase-treated-fortified, 4) 2-week old amylase-treated-fortified, 5) 6-month old non-amylase-treated-fortified, and 6) 2-week non-amylase-treated-fortified.

[2] column values with the same letter superscript were not significantly different by paired t-test at $p < 0.05$.

[3] the observed microbial load (total plate count) was within the acceptable level of 5000 cfu/g (Codex, 1981).

[4] moisture content and water activity measure water availability for chemical reactions and microbial growth and toxin production (e.g. aflatoxins); peroxide value is a measure of lipid oxidation; total plate count and yeasts and moulds indicate product hygiene; and aroma, texture and appearance indicate human acceptance of a food product.

5.2.7 Aflatoxins in groundnuts and extrusion cooked blend

12% of the groundnuts purchased were visibly contaminated (broken, discolored, shriveled, and insect-damaged). This resulted in a 48% higher charge per kilogram of groundnuts at the market compared with unsorted groundnuts. The mean total aflatoxin content of the suspect groundnut kernels was 80.2 (SD 76) ppb (95% CI: 33, 127). Five (62.5%), 3 (37.5%), 5 (62.5%) and 2 (25%) of the suspect groundnut kernel samples (n = 8), respectively, were contaminated with aflatoxins B_1, B_2, G_1 and G_2. Table 5-9 shows levels (mean and 95% CI) of aflatoxins B_1, B_2, G_1 and G_2 in suspect groundnut kernels. Although the study was not designed to assess the effect of extrusion cooking on aflatoxin (the blend was processed from clean groundnuts after sorting), the low moisture content and water activity resulting from processing was expected to inhibit mould growth in storage.

Table 5-9 Levels of aflatoxins B_1, B_2, G_1 and G_2 in suspect groundnut kernels (broken, discolored, shriveled, and insect-damaged)

Aflatoxin	Mean (µg/kg)	95% CI
AFB_1	38.4	9.9, 66.8
AFB_2	3.1	2.6, 3.7
AFG_1	38.7	7.0, 70.5
AFG_2	3.1	1.6, 4.6

5.2.8 Cost of α-amylase-treated-fortified blend

Table 5-10 shows the cost analysis per tonne of extrusion cooked α-amylase-treated multi-micronutrient fortified maize-beans-bambaranuts-groundnuts blend. The fact the cost of mineral-vitamin premix; freight and customs charges were very high may be attributed to the fact the complementary food was produced on small scale and the importation of micronutrient premix. Future scale up of production may result in higher economies of scale.

Table 5-10 Cost analysis per tonne of extrusion cooked α-amylase-treated multi-micronutrient fortified maize-beans-bambaranuts-groundnuts blend.

Production input	Quantity	Unit price US$	Actual cost US$	% basic cost
Raw materials				
Maize	650 kg	0.17	109.5	7.8
Beans	150 kg	0.53	79.0	5.6
Groundnuts	150 kg	0.96	143.6	10.2
Bambaranuts	50 kg	0.96	47.9	3.4
α-amylase	0.4 kg	53.19	21.3	1.5
Vitamin-mineral premix	6.25 kg	50.00	312.5	22.3
Tricalciumphosphate	20 kg	1.86	37.2	2.7
Micronutrient freight			388.9	27.7
Customs charges			208.6	14.9
Man hours				<0.1
Ingredient assembling	3*0.3 hrs	0.49	0.4	<0.1
Grinding	2*0.3 hrs	0.49	0.3	<0.1
Mixing	2*0.41 hrs	0.49	0.4	<0.1
Extrusion	1*1.67 hrs	0.49	0.8	0.1
Additive mixing	2*0.41 hrs	0.49	0.4	<0.1
Packaging and sealing	3*5 hrs	0.49	7.4	0.5
Loading	3*0.5 hrs	0.49	0.7	0.1
Transport	1*1 hr	0.49	0.5	<0.1
Machine hours				<0.1
Grinding	0.3 hrs	0.12	0.0	<0.1
Mixing	0.41 hrs	0.19	0.1	<0.1
Extrusion	1.67 hrs	2.40	4.0	0.3
Packing and sealing	4 hrs		3.7	0.3
Packaging material	1000 packs	0.03	25.0	1.8
Transport	1 trip for 2T truck	9.36	9.4	0.7
Basic cost total			1401.7	
Indirect costs and losses	15% of basic cost		210.3	
Total cost			1611.9	
Profit Margin	15% of total cost		241.8	
GRAND TOTAL/TONNE			**1853.7**	**100**

5.3 *Randomized controlled trial*

A total of 116 mother-infant pairs were recruited into the study. 14 (24%) and 21 (36%) subjects were lost to follow-up in the CBMA and CBM groups, respectively. Two main reasons for this loss were relocation of residence and the fact that some fathers were opposed to their children receiving food from the clinic because they could afford to buy complementary foods from the shops. Two mothers cited dark porridge color as the cause of their dropout. Two infants died (from the CBMA group) during the 3 month follow up due to upper respiratory illnesses. Measurements were obtained from all of the 69 mother-infant pairs recruited into the control group. A total of 8 infants were not breastfed at 9 months of age, 3 (8.1%) and 5 (7.2%) infants from CBM and control groups, respectively. Although the 8 non-breastfed infants were included in the general analysis, separate analyses were performed for them and are discussed.

This section presents results mainly for 9 months due to the fact that a control group was only available when infants were 9 months old. Secondly, breast milk intake data were obtained when infants were 9 months old.

5.3.1 Demographic and baseline characteristics and asset ownership

Table 5-11 presents mean (standard deviation) infants' birth weight and selected household demographic characteristics (CBM: n = 37; CBMA: n = 44; control: n = 69; and dropouts from intervention: n = 35). There were no significant differences in infants' birth weight and most of the household demographic characteristics. Table 5-12 shows source of drinking water, type of housing and

household asset ownership. There were no differences in general socio-economic characteristics between groups.

Table 5-11 Infants' birth weight and household baseline characteristics

	CBM (n = 37)	CBMA (n = 44)	Control (n = 69)	Dropouts[1] (n = 35)	P
Birth weight (kg)	3.1 (SD 0.5)	3.2 (SD 0.5)	3.1 (SD 0.5)	3.0 (SD 0.3)	0.70
Mother's age	27.2 (SD 5.9)	27.2 (SD 4.7)	25.6 (SD 5.3)	25.2 (SD 5.3)	0.34
Marital status [n (%)]					0.60
Married	29 (83)	36 (82)	49 (71)	27 (85)	
Widow	1 (3)	2 (4.5)	0	0	
Single	5 (14.3)	6 (24)	18 (16)	5 (15)	
Divorced	0	0	1 (1.4)	0	
Mother's education [n (%)]					0.90
Primary	6 (17)	10 (23)	15 (22)	11 (31)	
Secondary	21 (60)	20 (46)	36 (52)	19 (54)	
Tertiary colle.g.e	6 (17)	14 (32)	16 (24)	5 (15)	
University	2 (6)	0	1 (1.4)	0	
Mother's occupation [n (%)]					0.25
Salaried employed	5 (14)	7 (16)	11 (16)	5 (15)	
Self-employed	5 (14)	7 (16)	6 (9)	0	
Housewife	22 (63)	25 (57)	35 (51)	24 (69)	
Other (student/dependant)	3 (9)	4 (9)	16 (23)	5 (15)	
Father's age	34.2 (SD 5.7)	33.6 (SD 5.5)	32.8 (SD 7.4)	33.6 (SD 5.7)	0.80
Father's education [n (%)]					0.87
Primary	1 (3)	3 (7)	2 (3)	3 (8)	
Secondary	14 (40)	17 (39)	26 (38)	18 (51)	
Tertiary colle.g.e	11 (31)	13 (30)	15 (22)	7 (20)	
University	4 (11.4)	5 (11.4)	5 (7.2)	5 (15)	
Father's occupation [n (%)]					0.67
Salaried employed	23 (77)	30 (79)	35 (71)		
Self-employed	4 (13)	7 (18)	10 (20)		
Other	0	0	1 (2)		
Household occupancy					
Number of persons	6.1 (SD 2.2)	6.9 (SD 2.7)	6.6 (SD 3.2)	6.0 (SD 2.0)	0.55
Number of children	3.0 (SD 1.9)	2.9 (SD 1.5)	3.0 (SD 1.7)	3.0 (SD 1.6)	0.97

[1] dropouts from combined intervention groups (n = 21 from CBM and n = 14 from CBMA).

Table 5-12 Source of drinking water, type of housing and household asset ownership

Variable	CBM (n = 37)	CBMA (n = 44)	Control (n = 69)	Dropouts (n = 35)	P
Electricity	30 (85.7)	40 (90.9)	56 (81.2)	32 (91)	0.4
Solar power	1 (2.9)	1 (2.3)	1 (1.4)	0 (100)	0.9
Radio	29 (82.9)	40 (90.9)	62 (89.9)	30 (85)	0.5
Television	30 (85.7)	36 (81.8)	57 (82.6)	30 (85)	0.9
Telephone (landline/mobile)	25 (71.4)	27 (61.4)	32 (46.4)	23 (65)	0.05
Refrigerator	25 (71.4)	30 (68.2)	43 (62.3)	19 (55)	0.7
Bicycle	11 (31)	7 (16)	17 (24.6)	13 (36)	0.3
Motorcycle	0	1 (2.3)	3 (4.3)	0	0.6
Motor car	7 (20.0)	8 (18.2)	9 (13.0)	4 (10)	0.8
Source of water					0.6
Tap	34 (97.1)	43 (97.7)	67 (97.1)	32 (91)	
Borehole	1 (2.9)	1 (2.3)	2 (2.9)		
Type of housing					0.5
Low density	9 (25.7)	9 (20.5)	12 (17.4)	8 (22)	
Medium density	18 (51.4)	25 (56.8)	39 (56.5)	18 (50)	
High density	8 (22.9)	10 (22.7)	17 (24.6)	7 (18)	
Flooring material					0.5
Earth/mud/dung	1 (2.9)	0	1 (1.4)		
Ceramic tiles	2 (5.7)	1 (2.3)	3 (4.3)		
Concrete/cement	32 (91.4)	42 (95.5)	64 (92.8)	35 (100)	
Carpet	0	1 (2.3)	1 (1.4)		

5.3.2 Morbidity

Table 5-13 summarizes infant morbidity data for the month and 3 days immediately preceding the interviews at 9 months of age. A higher proportion of infants in the control group were reported to have been ill in the past month at 9 months compared to the two intervention groups. However, a higher proportion of infants in the two intervention groups sought treatment at the clinic, likely, due to increased attention and care they received from nurses involved in the study. This discrepancy may be due to the greater contact of mothers in the intervention groups with the project staff which might have encouraged them to attend clinic.

There were no differences between intervention groups and control in morbidity in the last 3 days at 9 months although a slightly higher proportion of infants had runny noses in the latter group.

Table 5-13 Infant morbidity over the last month and 3 days preceding interview

	CBM (n = 37)	CBMA (n = 44)	Control (n = 69)	P
State of health in past month [n (%)]				
Generally well	20 (55)	24 (54)	54 (78)	0.07
Mild-self-limiting illness	10 (28)	7 (15)	10 (15)	0.90
Ill and treated at clinic	5 (14)	13 (29)	5 (7)	0.90
Hospitalization	1 (3)	1 (2)	0	-
Morbidity in the last 3 days [n (%)]				
Diarrhea	3 (8)	4 (9)	7 (10)	0.98
Vomiting	2 (6)	1 (3)	3 (4)	0.70
Fever	5 (14)	7 (16)	9 (13)	0.90
Cough	9 (24)	6 (14)	17 (20)	0.26
Wheezing	1 (3)	0	1 (1)	0.25
Running nose	6 (16)	9 (20)	20 (30)	0.38
Ear problems	0	0	0	
Skin problems	4 (12)	4 (9)	11 (16)	0.57
Excessive crying	1 (3)	1 (3)	3 (4)	0.84
Eating problems	5 (14)	4 (9)	6 (8)	0.59

5.3.3 Growth and haemoglobin concentration

5.3.3.1 Anthropometric measurements

5.3.3.1.1 Growth

The mean weight at 6 months was 7.8 (SD 0.8) kg, 7.9 (SD 0.9) kg and 7.9 (SD 1.0) kg for infants in the CBM, CBMA and control groups, respectively. The mean weight at 9 months was 9.0 (SD 1.5) kg, 8.9 (SD 1.4) kg and 8.6 (SD 1.1) kg for infants in the CBM, CBMA and control groups, respectively. There were no significant differences in weight among the groups at 6 and 9 months. The

mean weight gain between 6 and 9 months was 1.0 (SD 0.6) kg, 0.9 (SD 0.6) kg and 0.9 (SD 0.5) kg for infants in the CBM, CBMA and control groups, respectively ($p = 0.54$).

The mean length at 6 months was 67.2 (SD 2.7) cm and 67.4 (SD 2.5) for infants in the CBM and CBMA, respectively. There was no significant difference in length between the CBM and CBMA at 6 months. The mean length at 9 months was 71.8 (SD 2.5) cm, 71.3 (SD 1.5) cm and 70.9 (SD 2.4) cm for infants in the CBM, CBMA and control groups, respectively. The mean length gain between 6 and 9 months was 4.5 (SD 2.0) cm and 4.2 (1.4) cm in the in the CBM than CBMA groups, respectively ($p = 0.22$). Data on length gain was not available for the control group as length at 6 months was not measured. There was a trend ($p = 0.06$) towards greater length in CBM and CBMA than control groups. Infants in CBM had significantly greater length ($p = 0.04$) at 9 months than infants in the control group. The prevalence of stunting (HAZ < -2 SD) was low in both CBM and CBMA at baseline (2.7% and 2.2%, respectively) and at 9 months in both intervention groups and controls (2.7%, 2.2% and 1.4%, for CBM, CBMA and controls, respectively). The overall length gain between 6 and 9 months was higher by 0.7 cm in CBM than in CBMA. Figure 5-2 shows length-for-age (HAZ) at 6 and 9 months for the study groups. There were no significant differences in HAZ between CBM and CBMA at 6 and 9 months. Infants in CBM had significantly greater HAZ ($p = 0.03$) at 9 months than infants in the control group. Figure 5-3 shows the weight-for-age growth curves for CBM, CBMA and control infants between 6 and 9 months. There were no significant differences in weight-for-age Z-score (WAZ) among the three groups at any time point. There was a

decline in WAZ in all three groups over the 3-month period; however, the rates of decline were not significantly different.

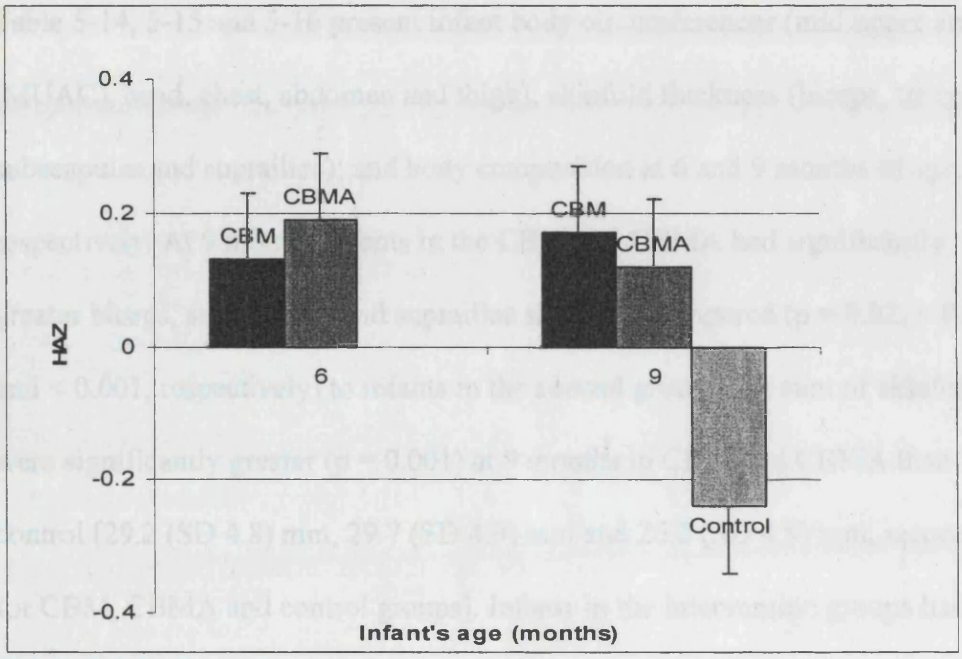


Figure 5-2 Length-for-age Z-scores (HAZ) for infants at 6 and 9 months of age

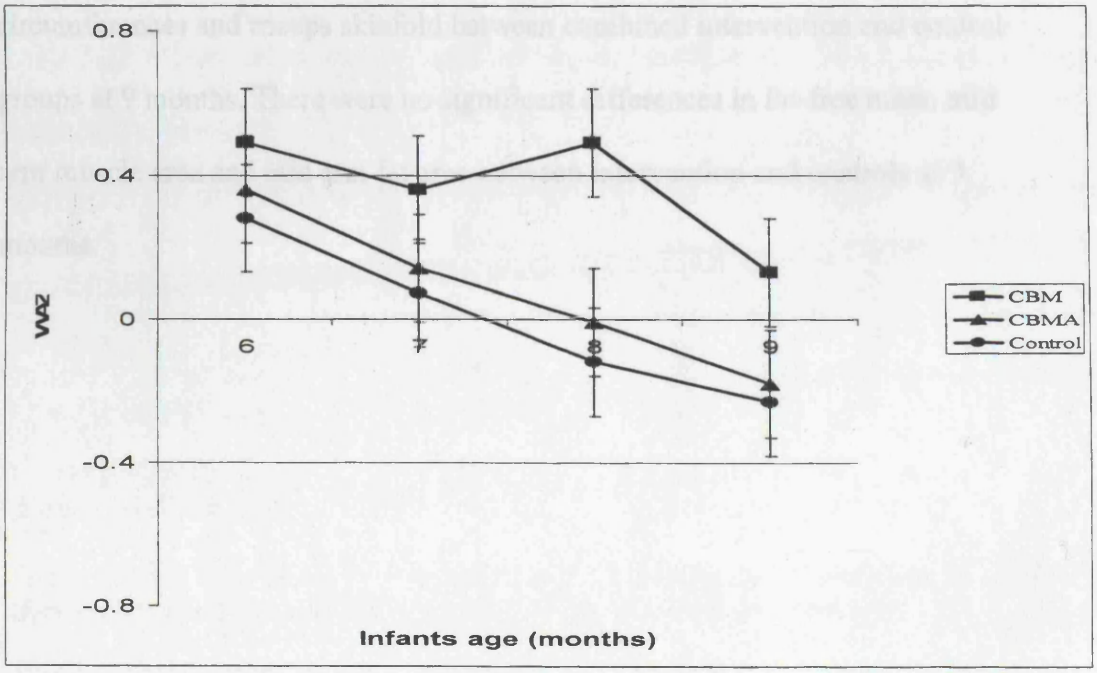


Figure 5-3 Weight-for-age (WAZ) growth curves for CBM, CBMA and control infants between 6 and 9 months

5.3.3.1.2 Body composition

Table 5-14, 5-15 and 5-16 present infant body circumferences (mid upper arm (MUAC), head, chest, abdomen and thigh); skinfold thickness (biceps, triceps, subscapular and suprailiac); and body composition at 6 and 9 months of age, respectively. At 9 months infants in the CBM and CBMA had significantly greater biceps, subscapular and suprailiac skinfolds compared ($p = 0.02$, < 0.001, and < 0.001, respectively) to infants in the control group. The sum of skinfolds were significantly greater ($p = 0.001$) at 9 months in CBM and CBMA than in control [29.2 (SD 4.8) mm, 29.7 (SD 4.9) mm and 26.2 (SD 4.9) mm, respectively for CBM, CBMA and control groups]. Infants in the intervention groups had significantly greater fat mass than control infants. There were no significant differences in mid upper arm circumference (MUAC), head, chest, and thigh circumferences and triceps skinfold between combined intervention and control groups at 9 months. There were no significant differences in fat-free mass, mid arm muscle area and mid arm fat area between intervention and controls at 9 months.

Table 5-14 Infant body circumferences at 6 and 9 months of age

	CBM (n = 37)	CBMA (n = 44)	Control (n = 69)	P
Body circumference				
MUAC at 6 months(cm)	14.5 (SD 1.3)	14.4 (SD 1.2)	Not measured	0.48
MUAC at 9 months(cm)	15.2 (SD 1.5)	14.7 (SD 1.3)	14.7 (SD 1.2)	0.18
Head at 6 months (cm)	43.4 (SD 1.8)	43.3 (SD 1.7)	Not measured	0.88
Head at 9 months (cm)	45.2 (SD 1.6)	44.9 (SD 1.5)	44.8 (SD 1.4)	0.38
Chest at 6 months (cm)	43.1 (SD 4.1)	43.6 (SD 2.7)	Not measured	0.51
Chest at 9 months (cm)	45.5 (SD 3.0)	45.8 (SD 3.2)	45.2 (SD 2.5)	0.48
Abdomen at 6 months (cm)	45.8 (SD 3.8)	45.4 (SD 3.2)	Not measured	0.63
Abdomen at 9 months (cm)	48.2 (SD 3.9)	48.1 (SD 3.7)	46.6 (SD 3.9)	0.05
Thigh at 6 months (cm)	23.7 (SD 2.9)	23.1 (SD 2.8)	Not measured	0.33
Thigh at 9 months (cm)	24.7 (SD 3.1)	24.8 (SD 3.6)	24.0 (SD 3.5)	0.77

Table 5-15 Infant biceps, triceps, subscapular and suprailiac skinfold thickness at 6 and 9 months of age[1].

	CBM (n = 37)	CBMA (n = 44)	Control (n = 69)	P
Skinfold				
Biceps at 6 months (mm)	6.0 (SD 1.2)	6.1 (SD 1.3)	Not measured	0.70
Biceps at 9 months (mm)	6.5 (SD1.4)[a]	6.6 (SD 1.3)[a]	5.9 (SD 1.4)[b]	0.02
Triceps at 6 months (mm)	8.3 (SD 1.7)	8.2 (SD 1.5)	Not measured	0.89
Triceps at 9 months (mm)	8.9 (SD 1.8)	9.1 (SD 1.7)	8.7 (SD 1.9)	0.51
Subscapular at 6 months (mm)	9.2 (SD 2.0)	8.9 (SD 1.7)	Not measured	0.60
Subscapular at 9 months(mm)	9.4 (SD 2.4)[a]	9.5 (SD 2.1)[a]	8.2 (SD 1.7)[b]	<0.001
Suprailiac at 6 months (mm)	4.9 (SD 1.3)	4.9 (SD 1.8)	Not measured	0.95
Suprailiac at 9 months (mm)	5.1 (SD 1.3)[a]	5.0 (SD 1.0)[a]	3.8 (SD 0.9)[b]	<0.001

[1] row values bearing the same letter superscript were not significantly different at $p < 0.05$ by one –way ANOVA and least square difference.

Table 5-16 Infant body composition at 6 and 9 months of age[1]

	CBM (n = 37)	CBMA (n = 44)	Control (n = 69)	P
Fat Mass at 6 months (%)	22.5 (SD 2.4)	22.4 (SD 2.4)	Not measured	0.85
Fat Mass at 9 months (%)	23.2 (SD 2.7)[a]	23.4 (SD 2.5)[a]	21.6 (SD 2.6)[b]	0.01
Fat Free Mass at 6 months (kg)	6.2 (SD 0.9)	6.2 (SD 0.8)	Not measured	0.95
Fat Free Mass at 9 months (kg)	6.9 (SD 0.9)	6.8 (SD 0.9)	6.9 (SD 0.8)	0.73
Mid Arm Muscle Area 6 months (mm^2)	1146 (SD 223)	1132 (SD 368)	Not measured	0.40
Mid Arm Muscle Area 9 months (mm^2)	1236 (SD 234)	1174 (SD 235)	1184 (SD 232)	0.27
Mid Arm Fat Area (MAFA) 6 months (mm^2)	552 (SD 144)	541 (SD 130)	Not measured	0.46
Mid Arm Fat Area (MAFA) 9 months (mm^2)	622 (SD 163)	621 (SD 161)	584 (SD 147)	0.35

[1] row values bearing the same superscript were not significantly different at $p < 0.05$ by one –way ANOVA and least square differences

5.3.3.1.3 Maternal anthropometry

Table 5-17 shows maternal weight, height, body mass index and body composition at 6 and 9 months. There were no significant differences in any maternal anthropometric indices between CBM and CBMA at 6 months. There were significant differences in most of maternal anthropometric indices between intervention and control groups at 9 months. Mothers in CBM and CBMA had greater suprailiac skinfold than mothers in the control group at 9 months.

Table 5-17 Maternal weight, height, body mass index (BMI) and body composition at 6 and 9 months[1]

	6 months			9 months			
	CBM (n = 37)	CBMA (n = 44)	P	CBM (n = 37)	CBMA (n = 44)	Control (n = 69)	P
Weight (kg)	60.1 (SD 13.5)	60.6 (SD 13.2)	0.89	60.3 (SD 14.5)	60.1 (SD 14.4)	58.5 (SD 9.6)	0.71
Height (cm)	159.2 (SD 5.3)	161.5 (SD 5.9)	0.09	159.0 (SD 5.2)	161.5 (SD 5.9)	158.9 (SD 6.1)	0.06
BMI (kg/m^2)	23.8 (SD 4.9)	23.3 (SD 4.6)	0.67	23.8 (SD 5.1)	22.9 (SD 4.9)	23.1 (SD 3.6)	0.70
Body circumference							
MUAC (cm)	27.6 (SD 4.4)	27.1 (SD 4.3)	0.63	27.9 (SD 4.9)	27.0 (SD 4.4)	26.9 (SD 3.3)	0.63
Head (cm)	57.1 (SD 2.5)	57.2 (SD 2.0)	0.87	57.4 (SD 2.2)	57.3 (SD 1.9)	56.9 (SD 2.6)	0.45
Chest (cm)	85.2 (SD 10.3)	85.4 (SD 8.3)	0.91	86.0 (SD 10.8)	85.6 (SD 8.7)	85.1 (SD 6.5)	0.86
Abdomen (cm)	78.7 (SD 10.8)	77.8 (SD 11.1)	0.72	79.6 (SD 11.5)	78.0 (SD 11.7)	78.3 (SD 8.6)	0.78
Thigh (cm)	50.8 (SD 7.3)	50.8 (SD 7.0)	0.97	51.0 (SD 8.4)	50.9 (SD 7.2)	49.7 (SD 8.9)	0.67
Skinfold thickness							
Biceps (mm)	7.8 (SD 3.6)	7.9 (SD 4.5)	0.90	8.4 (SD 4.2)	7.9 (SD 3.9)	7.2 (SD 3.4)	0.30
Triceps (mm)	13.2 (SD 5.2)	13.2 (SD 4.7)	0.95	14.9 (SD 6.3)	13.5 (SD 4.8)	14.7 (SD 6.3)	0.47
Subscapular (mm)	15.5 (SD 8.1)	14.9 (SD 7.0)	0.75	15.6 (SD 8.1)	15.0 (SD 7.1)	13.1 (SD 6.4)	0.18
Suprailiac (mm)	6.5 (SD 2.9)	6.2 (SD 3.4)	0.68	6.5 (SD 2.8)[a]	6.3 (SD 3.0)[a]	4.8 (SD 1.4)[b]	< 0.001
Body composition							
Upper Arm Muscle Area (cm2)	28.3 (SD 36.4)	28.5 (SD 39.1)	0.99	47.8 (SD 63.4)	30.6 (SD 41.5)	54.9 (SD 88.4)	0.23
Fat Mass (%)	27.5 (SD 5.9)	27.4 (SD 5.3)	0.91	28.3 (SD 6.1)	27.7 (SD 5.2)	26.7 (SD 5.1)	0.35
Fat Free Mass (kg)	43.0 (SD 6.8)	43.5 (SD 7.0)	0.75	42.3 (SD 6.7)	42.9 (SD 7.6)	42.5 (SD 5.6)	0.95

[1] row values bearing the same superscript were not significantly different at $p < 0.05$ by one-way ANOVA and least square difference

5.3.3.2 Haemoglobin concentration

There was no significant difference in mean haemoglobin concentration at 6 months between infant and in the CBM and CBMA (10.8 (SD 1.2) g/dL and 10.2 (SD 1.7) g/dL for CBM and CBMA, respectively). The mean haemoglobin concentration at 9 months was 10.4 (SD 1.2) g/dL, 10.3 (SD 1.2) g/dL and 9.8 (SD 1.4) g/dL for infants in the CBM, CBMA and control groups, respectively. Infants in both CBM and CBMA had significantly higher haemoglobin concentration ($p = 0.03$) than infants in the control group at 9 months. There were no significant differences in changes in haemoglobin concentration between intervention groups, however, infants in CBMA had overall higher gain in haemoglobin concentration (5 g/L) than infants in the CBM group. There were no significant differences in haemoglobin concentration at 9 months between intervention groups. There were no differences in all cate.g.ories of anemia prevalence between intervention groups at both 6 and 9 months. At 9 months control infants had significantly higher ($p = 0.01$) anemia prevalence (Hb < 110 g/L) compared to the intervention groups. Figure 5-4 shows the proportion of infants with haemoglobin concentration less than cut-off at 9 months of age.

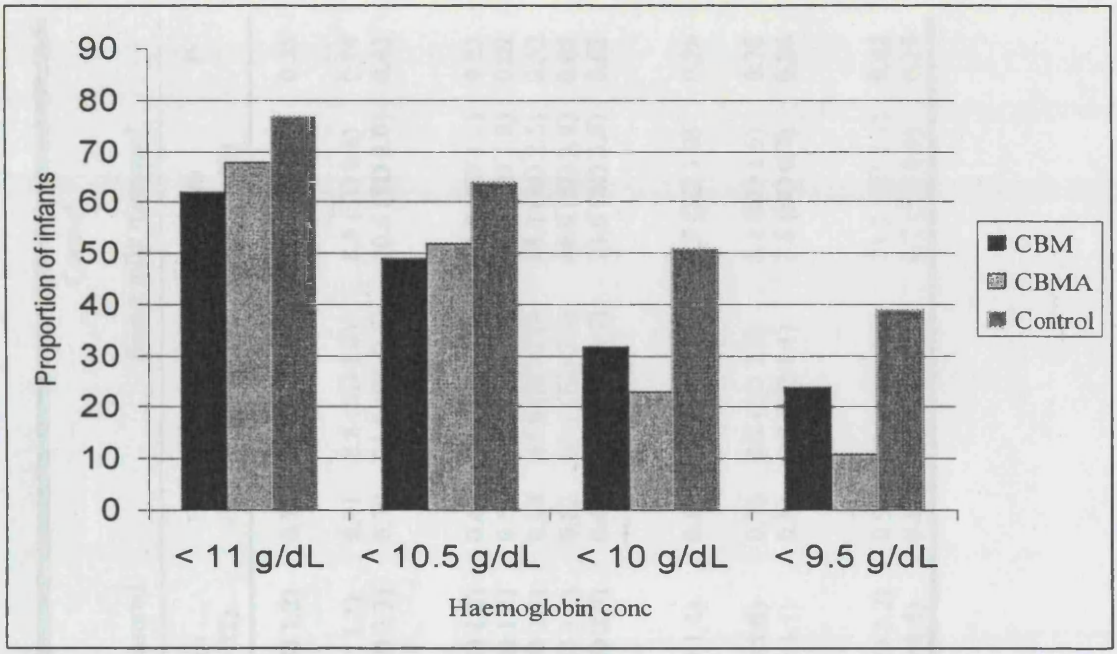


Figure 5-4 Proportion of infants with haemoglobin concentration less than cut-off at 9 months of age

5.3.4 Breast milk and nutrient intake

Table 5-18 presents a comparison by treatment group of the haemoglobin concentration and anthropometric indices at 9 months for infants in the breast milk measurement subset and not in the subset. There were no significant differences between the subset of infants whose breast milk intake was measured and the rest of the infants in all the groups, except in the control group where infants in the breast milk subset had significantly greater head circumference. Hence, the average breast milk values obtained for each group subset were used to calculate total nutrient intake for the entire CBM, CBMA and control groups.

Table 5-18 Comparison by treatment group of the haemoglobin concentration and anthropometric indices at 9 months for infants in and without breast milk measurement subset

Variable	CBM			CBMA			Control		
	Breast milk measured			Breast milk measured			Breast milk measured		
	Yes (n = 14)	No (n = 23)	P	Yes (n = 12)	No (n = 32)	P	Yes (n = 27)	No (n = 42)	P
Hemoglobin (g/dL)	10.6 (SD 1.5)	10.3 (SD 1.1)	0.49	10.6 (SD 1.0)	10.1 (SD 1.2)	0.15	10.0 (SD 1.3)	9.6 (SD 1.5)	0.25
Weight (kg)	9.0 (SD 1.6)	9.0 (SD 1.2)	0.86	8.7 (SD 1.2)	8.7 (SD 1.1)	0.91	8.8 (SD 1.0)	8.5 (SD 0.8)	0.14
Length (cm)	72.0 (SD 3.4)	71.7 (SD 2.6)	0.77	71.6 (SD 1.4)	71.4 (SD 2.3)	0.70	71.0 (SD 2.5)	70.5 (SD 2.0)	0.43
Circumferences (cm)									
MUAC	15.2 (SD 1.5)	15.1 (SD 1.5)	0.90	14.9 (SD 1.4)	14.7 (SD 1.2)	0.61	14.9 (SD 1.3)	14.5 (SD 1.2)	0.23
Head	45.5 (SD 1.4)	45.0 (SD 1.9)	0.37	45.0 (SD 1.3)	44.7 (SD 1.6)	0.55	45.1 (SD 1.4)	44.4 (SD 1.0)	0.02
Chest	45.4 (SD 2.4)	45.3 (SD 2.9)	0.86	45.6 (SD 2.8)	45.5 (SD 2.7)	0.94	44.8 (SD 2.0)	45.1 (SD 2.5)	0.52
Abdomen	48.3 (SD 4.4)	47.7 (SD 3.1)	0.63	47.6 (SD 3.5)	47.9 (SD 3.1)	0.82	45.1 (SD 3.1)	46.9 (SD 3.7)	0.05
Thigh	25.0 (SD 3.4)	24.5 (SD 2.9)	0.64	24.8 (SD 3.6)	24.3 (SD 2.7)	0.61	24.0 (SD 2.7)	23.6 (SD 3.8)	0.63
Skinfolds (mm)									
Biceps	6.8 (SD 1.5)	6.3 (SD 1.0)	0.18	6.4 (SD 1.2)	6.5 (SD 1.4)	0.86	6.1 (SD 1.8)	5.7 (SD 1.0)	0.26
Triceps									
Subscapular	9.1 (SD 2.6)	9.3 (SD 1.7)	0.75	9.2 (SD 2.4)	9.4 (SD 1.6)	0.78	8.2 (SD 2.0)	8.1 (SD 1.6)	0.78
Suprailiac	5.0 (SD 1.3)	5.0 (SD 1.2)	0.93	4.8 (SD 1.5)	5.0 (SD 1.1)	0.81	3.9 (SD 1.1)	3.6 (SD 0.7)	0.26
Body composition									
% Fat mass	21.3 (SD 5.9)	23.2 (SD 2.0)	0.21	23.3 (SD 2.6)	23.2 (SD 2.2)	0.93	21.7 (SD 2.8)	21.2 (SD 2.4)	0.42
Fat Free Mass (kg)	6.5 (SD 0.7)	6.9 (SD 0.8)	0.12	6.6 (SD 0.7)	6.7 (SD 0.8)	0.84	6.9 (SD 0.7)	6.7 (SD 0.6)	0.18

5.3.4.1 Breast milk intake

Table 5-19 presents the daily breast milk intake for infants in CBM, CBMA, and control groups at 9 months of age. There were no significant differences in daily breast milk intake and non-milk water intake among the three groups.

Table 5-19 Daily (mean, 95% confidence interval) breast milk, non-milk water intake and total water output by treatment of infants at 9 months of age.

	CBM (n = 14)	CBMA (n = 12)	Control (n = 27)	P
Breast milk (g/d)	614 (458, 771)	635 (512, 758)	653 (566, 741)	0.87
Non-milk water intake (ml/d)	451 (196, 705)	484 (204, 764)	434 (317, 551)	0.93
Total water output (ml/d)	1080 (870, 1290)	1129 (886, 1372)	1103 (1008, 1198)	0.92

5.3.4.2 Nutrient intake

Table 5-20 shows the total daily energy, protein, fat and fibre intake from breast milk and complementary foods and energy composition for infants at 9 months of age. There were no significant differences among the groups in energy, protein, fat and fibre intake. The proportion of energy intake contributed by protein was only slightly greater than the currently recommended value of 6-10% (Lutter and Dewey, 2003). The proportion of energy from fat was slightly higher than the

currently recommended value of 24% for infants aged 9-11 months (Lutter and Dewey, 2003).

Table 5-20 Daily mean (95% CI) intake of macronutrients from breast milk[1] and complementary foods and energy composition at 9 months.

Nutrient	CBM (n = 37) Intake from Breast milk	CBM (n = 37) Intake from solid foods	CBM % RDA	CBMA (n = 44) Intake from breast milk	CBMA (n = 44) Intake from solid foods	CBMA % RDA	Controls (n = 59) Intake from breast milk	Controls (n = 59) Intake from solid foods	Controls % RDA	P[2]
Energy (kcal)	412 (307, 516)	390 (315, 467)	116	425 (343, 508)	344 (267, 422)	112	438 (379, 496)	353 (299, 407)	115	0.63
Protein (g)	8.6 (6.4, 10.8)	13.7 (10.4, 17.1)	232	9.0 (6.8, 11.1)	10.5 (8.4, 12.6)	203	9.1 (7.8, 10.4)	11.5 (9.6, 13.5)	214	0.21
Fat (g)	11.1 (8.2, 13.9)	14.2 (9.0, 19.5)		11.5 (8.8, 14.3)	11.2 (7.8. 14.5)		11.7 (10, 13.4)	11.6 (9.5, 13.5)		0.43
Carbohydrate (g)	44.8 (33.4, 56.2)	63.4 (50.4, 76.5)		46.8 (35.7, 57.9)	53.0 (42.3, 63.3)		47.5 (40.5, 54.4)	53.9 (46.1, 61.8)		0.31
Fibre (g)	0	10.1 (3.2, 16.9)		0	5.4 (3.9, 6.9)		0	12.4 (6.2, 18.6)		0.24
Energy composition (%)[1]										
Protein	11.9 (11.4, 12.5)			11.2 (10.8, 11.6)			11.8 (11.0, 12.5)			0.76
Fat	28.9 (27, 30.7)			29.4 (26, 32.8)			28 (26, 30)			0.65
Carbohydrates	59.2 (57.3, 61)			59.4 (56.1, 62.7)			60.3 (57.9, 62.6)			0.45

[1] based on average breast milk intake (614 g/d, 635 g/d and 653 g/d for infants in the CBM, CBMA and control groups, respectively).
[2] since there were no significant differences among groups in breast milk intake, total differences are based on nutrient intake from complementaryfoods (row values with same letter superscripts were not significantly different at p = 0.05 by one-way ANOVA)

5.3.4.2.1 Net protein utilization

Table 5-21 shows Protein Efficiency Ration (PER), net protein utilization (NPU), total protein intake and weight gain between 6 and 9 months for CBM and CBMA.

Table 5-21 Protein Efficiency Rationn (PER), Net Protein Utilization (NPU), protein intake and weight gain between 6 and 9 months of age.

	CBM	CBMA
PER	2.9	3.1
NPU[1]	76.7	79.3
Protein intake (g)	343.5	256
Weight gain (g)	1000	800

[1]The NPU for CBM and CBMA observed in the current study are higher than the estimated average value, 69.1 (SD 12) for 12 West African complementary foods containing groundnuts, cereals (maize, millet or sorghum) and another legume (Onofiok and Nnanyelugo, 1998).

Table 5-22 presents the daily intake of minerals from breast milk and complementary foods and phytate content of diet 9 months of age. Infants in combined CBM and CBMA had significantly greater intake of calcium, iron and zinc than infants in the control group. Infants in the control had significantly ($p < 0.001$) greater phytate:zinc molar ratio and significantly ($p < 0.001$) greater phytate:iron molar ration than infats in the CBM and CBMA. There were no significant differences in phytate intake among the groups. Table 5-23 shows the daily intake vitamins from breast milk and complementary foods at 9 months of age. Infants in both CBM and CBMA had significantly greater vitamin A, vitamin

C and riboflavin intake and had a trend towards greater niacin intake than infants in the control group. Infants in the CBMA had significantly ($p = 0.03$) lower thiamine intake than infants in the CBM and control groups. There were no significant differences in pyridoxine intake among the groups. The observed trend for infants in the CBM to have higher micronutrient intakes than infants in CBMA may be explained by the fact that Chilenje Baby Mix and commercial complementary foods, some of which are fortified, comprised a greater proportion of daily nutrient intake in the former group (see Figure 5-5).

Table 5-22 Daily intake of minerals from breast milk[1] and complementary foods and phytate content of diet 9 months of age

Nutrient	CBM (n = 14) Intake from Breast milk	CBM (n = 14) Intake from solid foods	% RDA[2]	CBMA (n = 12) Intake from breast milk	CBMA (n = 12) Intake from Solid foods	% RDA[2]	Controls (n = 27) Intake from breast milk	Controls (n = 27) Intake from solid foods	% RDA[2]	P[3]
Calcium (mg)	129 (96, 162)	266[a] (167, 365)	99	135 (103, 166)	179[a] (114, 243)	79	137 (117, 156)	127[b] (86, 167)	66	0.01
Iron (mg)	0.22 (0.16, 0.27	10.2[a] (7.4, 13.0)	109	0.22 (0.12, 0.28)	8.0[a] (5.6, 10.5)	88	0.23 (0.19, 0.26)	4.3[b] (3.5, 5.1)	49	<0.001
Zinc (mg)	0.92 (0.7, 1.2)	3.6[a] (2.8, 4.5)	110	0.96 (0.7, 1.2)	2.9[a] (2.3, 3.6)	94	0.98 (0.8, 1.1)	2.2[b] (1.9, 2.6)	78	0.002
Phytate (mg)	0	284 (217, 351)		0	238 (194, 282)		0	284 (234, 334)		0.43
Phytate:zinc molar ratio	0	9.7[a] (7.6, 11.8)		0	10.2[a] (8.1, 12.3)		0	14.8[b] (12.7, 17)		<0.001
Phytate: iron molar ratio	0	4.1[a] (2.8, 5.4)		0	4.4 [a] (3.2, 5.6)		0	7.5[b] (6.2, 8.8)		<0.001

[1] based on average breast milk intake (614 g/d, 635 g/d and 653 g/d for infants in the CBM, CBMA and control groups, respectively).
[2]Recommended Dietary Allowance (RDA) is used throughout this thesis to compare infant nutrient intakes to requirements to allow for comparison of this study with other studies on complementary feeding of infants. Recommended Nutrient Intake (RNI) derived by factorial method is the United Kingdom equivalent of RDA. RDAs for infants' calcium and vitamin A intakes are lower than RNI for the two micronutrients (Lutter and Dewey, 2003). RDAs for B-vitamins are similar to the RNIs except for folate recommendation that is lower with RNI (Lutter and Dewey, 2003).
[3] since there were no significant differences among groups in breast milk intake, total differences are based on nutrient intake from complementary foods (row values with same letter superscripts were not significantly different at p = 0.05 by one-way ANOVA).

Table 5-23 Daily intake vitamins from breast milk[1] and complementary foods at 9 months of age

Nutrient	CBM (n = 14) Intake from breast milk	CBM (n = 14) Intake from solid foods	% RDA[2]	CBMA (n = 12) Intake from breast milk	CBMA (n = 12) Intake from solid foods	% RDA[2]	Controls (n = 27) Intake from breast milk	Controls (n = 27) Intake from solid foods	% RDA[2]	P[3]
Vitamin A (µg RE)	307 (229, 385)	278[a] (201, 356)	146	321 (245, 396)	194[a] (123, 265)	129	325 (278, 373)	153[b] (114, 192)	120	0.01
Vitamin C (mg)	28.9 (21.5, 36.2)	44.4[a] (29.6, 59.4)	244	30.1 (23, 37.2)	35.1[a] (21.8, 48.3)	217	30.6 (26.1, 35)	12.7[b] (7.7, 17.7)	144	<0.001
Thiamine (mg)	0.13 (0.1, 0.16)	393[a] (283, 502)	173	0.13 (0.1, 0.17)	267[b] (198, 337)	133	0.14 (0.12, 0.16)	273[a] (223, 324)	137	0.03
Riboflavin (mg)	0.22 (0.16, 0.27)	509[a] (311, 707)	182	0.22 (0.17, 0.28)	364[a] (260, 469)	145	0.23 (0.19, 0.26)	277[b] (206, 347)	128	0.02
Niacin (mg)	1.1 (0.8, 1.4)	4.9 (4.1, 5.8)	150	1.1 (0.9, 1.4)	4.2 (3.3, 5.1)	133	1.2 (1.0, 1.3)	3.7 (3.1, 4.3)	123	0.07
Pyridoxine (mg)	0.08 (0.06, 0.10)	0.68 (0.4, 1.0)	253	0.08 (0.06, 0.1)	0.74 (0.4, 1.1)	247	0.64 (0.31, 0.96	0.62 (0.4, 0.9)	207	0.84

[1] based on average breast milk intake (614 g/d, 635 g/d and 653 g/d for infants in the CBM, CBMA and control groups, respectively).
[2]Recommended Dietary Allowance (RDA) is used throughout this thesis to compare infant nutrient intakes to requirements to allow for comparison of this study with other studies on complementary feeding of infants. Recommended Nutrient Intake (RNI) derived by factorial method is the United Kingdom equivalent of RDA. RDAs for infants' calcium and vitamin A intakes are lower than RNI for the two micronutrients (Lutter and Dewey, 2003). RDAs for B-vitamins are similar to the RNIs except for folate recommendation that is lower with RNI (Lutter and Dewey, 2003).
[3] since there were no significant differences among groups in breast milk intake, total differences are based on nutrient intake from complementary foods (row values with same superscript were not significantly different at p = 0.05 by one-way ANOVA)

Figure 5-5 presents the percent contribution of different food groups to the daily energy intake from complementary foods of infants at 9 months of age. Infants in the control group received greater proportions of energy from cereals and cereal products, porridge with groundnuts and commercial complementary foods compared to infants in both intervention groups suggesting displacement of these food groups by the study blend. Infants in CBM had slightly greater proportions of their energy intake from Chilenje Baby Mix and commercial complementary foods at 9 months than infants in CBMA.

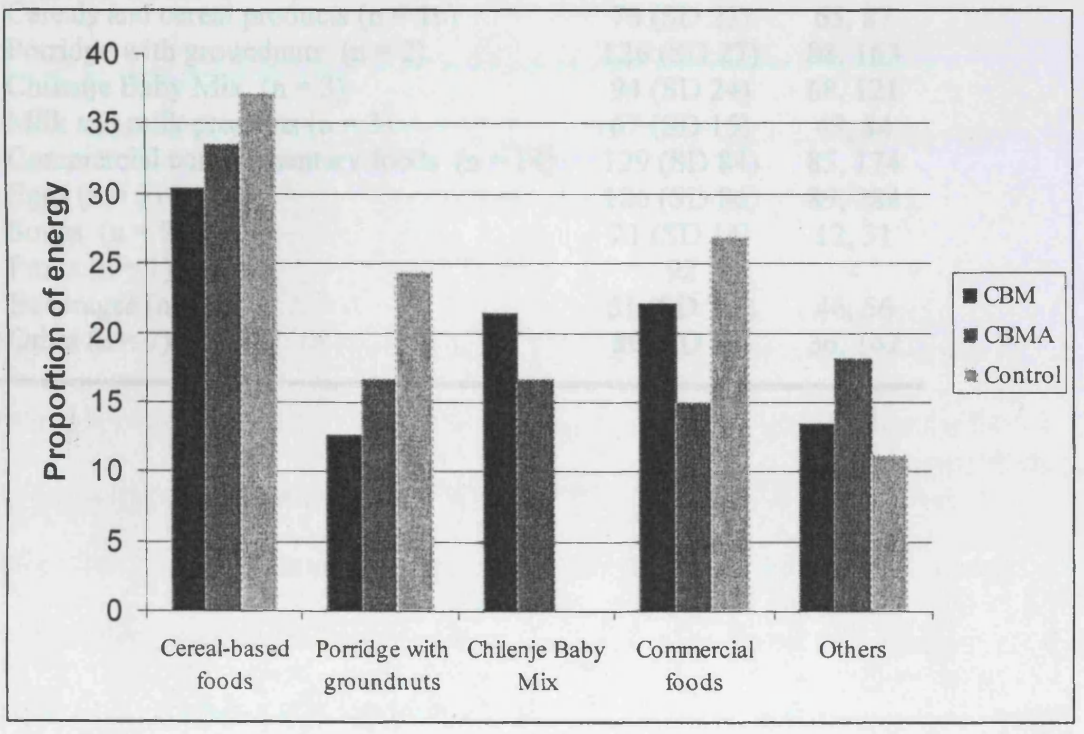


Figure 5-5 Proportion of energy intake from complementary foods obtained from different food groups at 9 months of age[1]

[1]Others include milk and milk products, e.g.gs, soups, beverages and other occassional foods.

Table 5-24 presents the energy densities of different food groups calculated based on 24-h recall data. The energy densities for three recipes for the study blend namely, Chilenje Baby Mix only, Chilenje Baby Mix with cooking oil and Chilenje Baby Mix with milk were 76, 86 and 121 kcal/100 g, respectively.

Table 5-24 Energy density (kcal/100g) for different food groups obtained from 24-h recall recipes.

	Energy Density	95% CI
Food group (number of recipes)		
Cereals and cereal products (n = 16)	76 (SD 23)	65, 87
Porridge with groundnuts (n = 2)	126 (SD 27)	88, 163
Chilenje Baby Mix (n = 3)	94 (SD 24)	68, 121
Milk and milk products (n = 3)	67 (SD 15)	49, 84
Commercial complementary foods (n = 14)	129 (SD 84)	85, 174
Eggs (n = 3)	186 (SD 86)	89, 284
Soups (n = 9)	21 (SD 14)	12, 31
Fruits (n = 1)	92	-
Beverages (n = 8)	51 (SD 7.3)	46, 56
Other (n = 7)	89 (SD 72)	36, 142

5.3.4.3 Influence of breast milk volume and breastfeeding status on infant growth, haemoglobin concentration and complementary feeding pattern

This sub-section presents secondary analyses that were not originally planned, but were necessitated by observed differences in complementary feeding pattern between breastfeeding and non-breastfeeding mothers.

5.3.4.3.1 Low versus average breast milk intake

A total of 10 infants (5 in CBM and CBMA and 5 in the control group had low mean breast milk intake, 278 (SD 120) g/d. 43 infants had average breast milk intake, 737 (SD 186) g/d. There were no significant differences in growth, haemoglobin concentration and body composition indices between infants with low breast milk intake compared to infants with average breast milk intake.

Figure 5-6 shows the proportion of energy obtained from different food groups by infants according to the level of breast milk intake. Infants with low breast milk intake received a higher proportion of energy from commercial complementary foods than infant with average breast milk intake. It is likely that breast milk was displaced by commercial complementary foods in the low breast milk intake group. It is apparent that breast milk still offered protection against growth faltering in the low breast milk intake group.

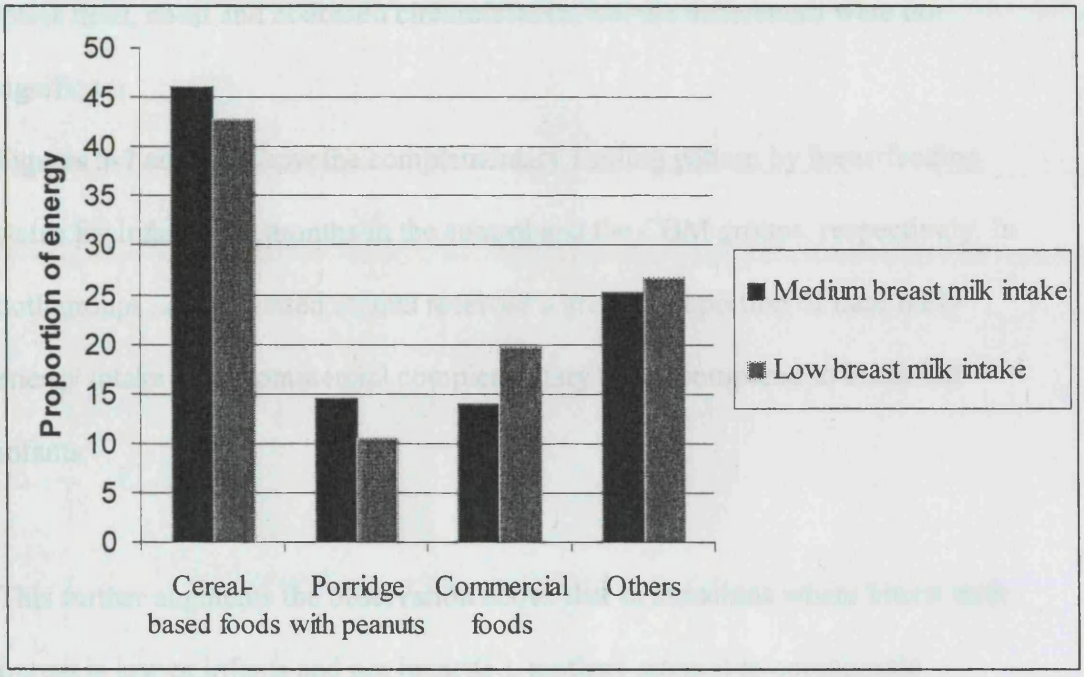


Figure 5-6 Infant complementary feeding pattern by breast milk intake level[1,2]

[1]Low breast milk intake in this study defined as daily milk intake < 450 g.
[2] Others include Chilenje Baby Mix, milk and milk products, beverages, soups, fruits, eggs and other occasional foods

5.3.4.3.2 Breastfed versus non-breastfed infants

There were a total of 8 (3 in CBM and 5 in the control group) non-breastfed infants in the study. Non-breastfed infants in the control group had significantly lower ($p = 0.04$) haemoglobin concentration than breastfed infants in that group (i.e. 8.5 (SD 1.9) g/dL versus 9.9 (SD 1.4) g/dL, respectively). There was no significant difference in haemoglobin concentration between breastfed and non-breastfed infants in the CBM group, however, the former had 5 g/L haemoglobin concentration higher than the latter. Although there were no significant differences in growth and body composition indices between non-breastfed and breastfed infants in the control group, the former had lower values for all the indices. Similarly, there was a trend for non-breastfed in the CBM group to have

lower head, chest and abdomen circumference, but the differences were not significant.

Figures 5-7 and 5-8 show the complementary feeding pattern by breastfeeding status for infants at 9 months in the control and the CBM groups, respectively. In both groups non-breastfed infants received a greater proportion of their daily energy intake from commercial complementary foods compared to breastfed infants.

This further augments the observation above that in situations where breast milk output is low or infants and not breastfed, mothers attempt to compensate apparent energy intake deficit by buying commercially available complementary foods. It is possible that mothers may not have enough money to buy adequate amounts of commercial complementary foods to meet entire daily requirements for infant growth and optimum haemoglobin concentration. This may explain the observation that non-breastfed infants in the control group had lower haemoglobin concentration levels than breastfed infants in the same group. The provision of Chilenje Baby Mix in the CBM group may have been beneficial in arresting decline in haemoglobin concentration levels in non-breastfed infants in that group.

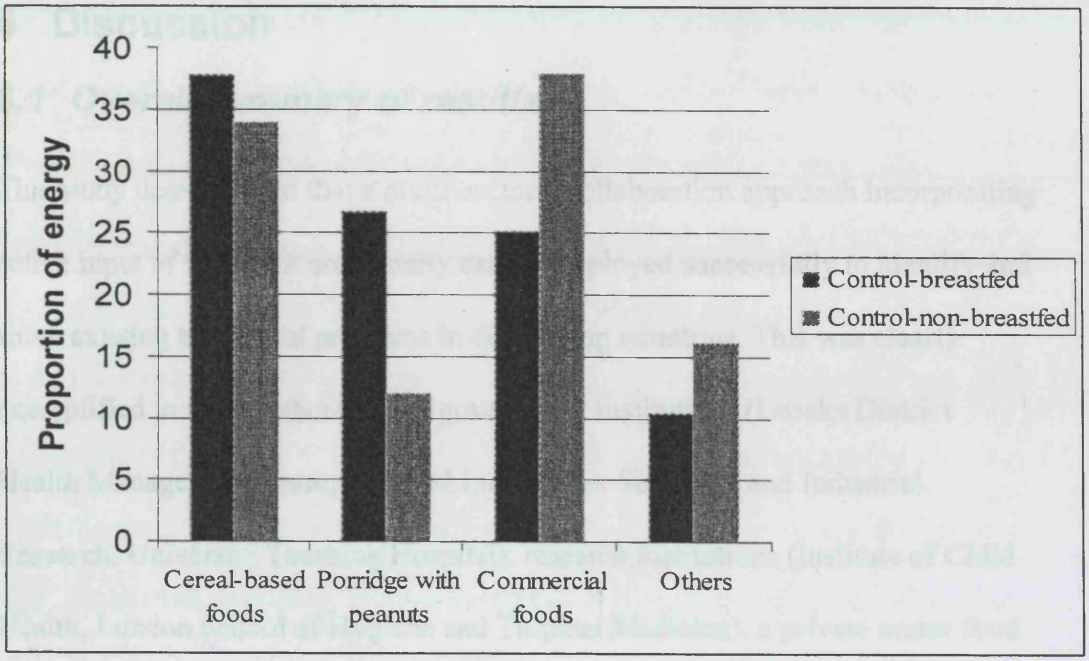


Figure 5-7 Infant complementary feeding pattern by breastfeeding status in control group

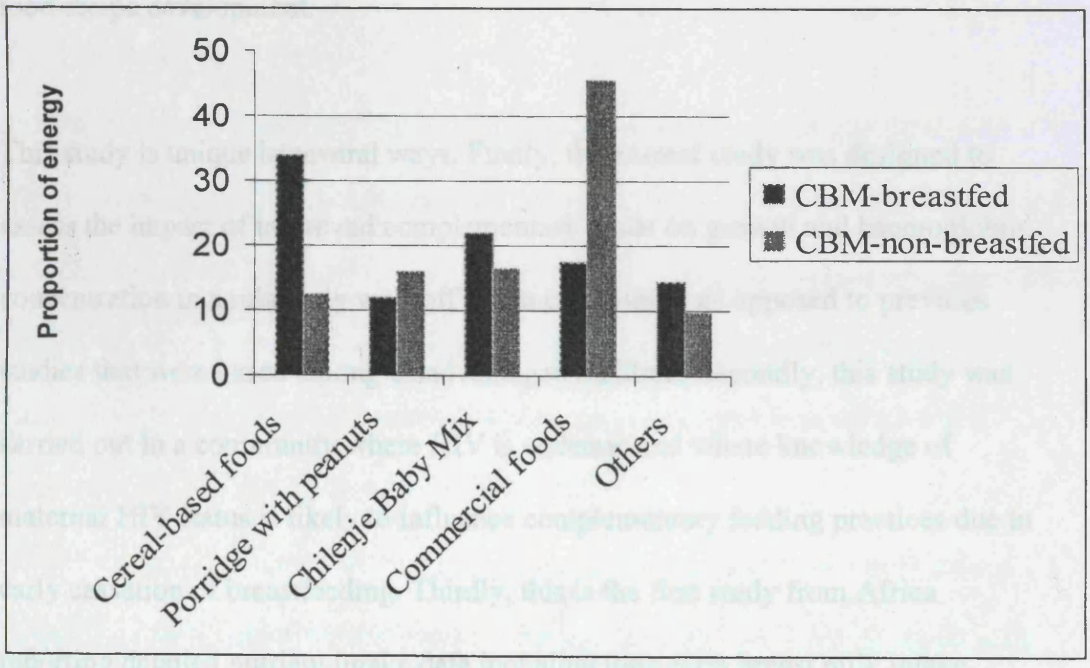


Figure 5-8 Infant complementary feeding pattern by breastfeeding status in the CBM group

6 Discussion

6.1 Overall summary of results

This study demonstrates that a multi-sectoral collaboration approach incorporating active input of the target community can be employed successfully to identify and solve existing nutritional problems in developing countries. This was clearly exemplified in the collaboration of government institutions (Lusaka District Health Management Team, National Institute for Scientific and Industrial Research, University Teaching Hospital), research institutions (Institute of Child Health, London School of Hygiene and Tropical Medicine), a private sector food processing company (Quality Commodities Limited) and involvement of the Chilenje Community Support Group (mothers, fathers and health workers) in initial problem identification and the specific input by mothers in complementary food recipe development.

This study is unique in several ways. Firstly, the current study was designed to assess the impact of improved complementary foods on growth and haemoglobin concentration in a relatively well-off urban community as opposed to previous studies that were based among disadvantaged children. Secondly, this study was carried out in a community where HIV is endemic and where knowledge of maternal HIV status is likely to influence complementary feeding practices due to early cessation of breastfeeding. Thirdly, this is the first study from Africa reporting detailed nutrient intake data including long-term breast milk intake measured over two weeks. Previous studies have reported breast milk intake based on test-weighing which is often inaccurate.

Overall, the results show that although Chilenje mothers were widely knowledgeable on sound breastfeeding and complementary feeding, their actual practices were constrained by time availability, maternal HIV status and availability of money to purchase food for their children. Secondly, although the intakes of macronutrients (energy, protein and fat) were not limiting, the intakes of important nutrients such as iron, calcium and zinc were suboptimal. The results further showed that it is feasible to produce a cheap, safe and acceptable complementary food based on the utilisation of widely accepted, already-in-use local cereals and le.g.umes. The results further showed that α-amylase enhanced porridge energy density and resulted in greater acceptability of porridges by mothers with minimal increase in cost. The results also show that micronutrient premix and associated freight and cost accounted for almost 60% of the total product cost.

Results from the randomized controlled trial show that there were no significant differences in total (from breast milk and complementary foods) energy, fat, protein, fibre, niacin, pyridoxine and phyate intakes; however the control group had significantly lower total intakes of calcium, iron, zinc, vitamin A, vitamin C, thiamine and riboflavin, and greater phytate:zinc and phytate:iron molar ratios compared to CBM and CBMA.

There were no significant differences between groups in daily breast milk intake suggesting that giving the study complementary foods did not displace breast milk intake.

The results show that consumption of fortified maize-beans-bambaranuts-groundnuts blends, re.g.ardless of amylase treatment, resulted in improvement in length and hemoglobin whereas weight gain was not affected. Infants in both intervention groups gained more fat. Breast milk intake did not differ between any of the groups and it is apparent that traditional complementary foods were displaced by the provision of higher quality complementary foods.

The results also show that non-breastfed infants in control group had significantly lower haemoglobin concentration than breastfed infants. Mothers with low breast milk output or those were not breastfeeding fed their infants with greater proportions of commercial complementary foods purchased from the shop.

6.2 Study limitations

6.2.1 Complementary development and production

Although the right amounts (based on recommended values and manufacturer's instructions) of mineral and vitamin premix was added to the blend and that thorough mixing was done during industrial production, it was not possible to successfully perform micronutrient analyses due to inadequate mineral and vitamin analysis capacity in Lusaka.

6.2.2 Randomized controlled trial

The loss of subjects (28%) was larger than the 15% had been assumed based on a previous study by the research group in the same population. Out of the total of 116 mother-infant pairs (58 mother-infant pairs in each of CBM and CBMA groups) who were recruited into the study and received the study complementary

blend at least once, 33 (21 and 12, from CBM and CBMA, respectively) mother-infant pairs were lost to follow up. 23 (69%) of the losses were due to the fact that the mother had moved from the original address recorded at recruitment or the recorded address could not be physically located. Two mothers indicated that they did not like the study blend, while one mother reported that her infant did not like the porridge. Four mothers reported that their husbands disallowed the feeding of the blend to their children. One mother was too busy at work to be available for follow up. Three infants died in the course of follow up.

Based on preliminary results that showed that energy intake was not limiting in Chilenje, it was postulated that iron deficiency anemia would be of more importance in this middle income urban community. Micronutrient deficiencies affect a much larger proportion of the population than those who have evident clinical symptoms and infants and children in both urban and rural settings are likely to be affected (Maberly et al., 1998). A sample size that would allow for detection of differences of at least 5 g/L in haemoglobin concentration (Lartey et al, 1999) was calculated. At 80% power and 5% significance we needed at least 58 infants per group. Allowing for 20% loss to follow up, a total of 70 infants were to be recruited per group. Differences of 6 g/L and 5 g/L between CBM and control and CBMA and control, respectively were observed in this study. Based on at 37 and 44 infants left in CBM and CBMA, we could detect 6.9 g/L and 6.3 g/L differences in haemoglobin concentration, respectively. Since the expected differences are comparable to the observed differences in this study, the large subject loss did not affect study conclusions.

The second limitation of the current study is the lack of baseline measurements of length, haemoglobin concentration, dietary intake and body composition indices for the control group. Baseline comparisons between intervention groups and the control are based on weight data that were obtained from growth charts for the latter group. Although historical events (Cook and Campbell, 1979) occurring between 6 and 9 months could have affected any observed differences between intervention and control groups, comparisons in this study are mainly confined to 9 months when the three groups were measured concurrently to minimise seasonal variation.

The other possible source of error in this study could have been the leakage of porridge to other members within the index infant's family or to families not in intervention. To prevent intra-household porridge leakage, the mothers were told to feed the porridge to the study child only and we provided an extra 2 kg per month of flour was provided to any mother with another child less than 3 years. Based on 24-h dietary recall, none of the mothers from the control group had fed their child with the study blend prior to recruitment. These mothers were provided with 2 months supply of flour after 2 week when all measurements had been done.

Nutrient intake data in this study may be limited by lack of adequate compliance data obtained based on mothers' monthly verbal report on the use of the blend. It was difficult for mothers to keep a food diary during this study.

Other shortcomings of the current study include the inability to assess factors such as aflatoxins, gut permeability and HIV infection that could confound growth and

haemoglobin concentration outcomes. These factors are discussed in detail later in section 6.3.3. The fact that infants who had symptoms of serious illness were excluded at 6 months in the intervention groups and at 8 months in the control group may have induced bias. It may be possible that infants in the intervention group developed disease symptoms between 6 and 9 months of age. Some infants in the intervention groups (n = 2) died. However, morbidity data show that there were no significant differences between intervention groups and control group, the infants in the latter group may have been slightly healthier.

To avoid any bias due to instrumentation, all measuring equipment were calibrated re.g.ularly. Weighing scales were calibrated weekly. Batteries for the Hemocue haemoglobin concentrationometer and the dietary scale were replaced every 2 months. Any inter-observer biases were minimized by ensuring only two trained nurses took all anthropometric measurements. Standardization of anthropometric measurments between the two trained nurses has been described above (see section 5.1.10).

6.3 Discussion of results

6.3.1 Complementary feeding practices and nutrient intake of children 6-18 months old

The dietary data show that energy and macronutrient intakes of infants and young children in this population were generally adequate, but that intakes of certain minerals, particularly iron, calcium and zinc, were inadequate. The strong support for breastfeeding by mothers, fathers and health workers contributed to the

adequate intakes of many nutrients. However, in order to improve infant nutrition in Lusaka, it may be necessary to develop and promote fortified complementary foods which are acceptable to and affordable by families.

The focus group discussion results show that even though some mothers used commercially processed complementary foods to feed their children, most mothers, including well educated people such as nurses, regard these foods with suspicion. This is mainly with regard to how long the foods have been on the shelf. Most of the existing commercially processed foods in Lusaka cost on average US$ 4 per kilogram pack. Most mothers in Chilenje could not afford these foods. Although the cost analysis for traditionally processed complementary foods was not done in the current study, it is expected that the long time spent by mothers in their preparation and losses during ingredient handling (e.g. sorting of groundnuts to remove discoloured, insect-damaged kernels) may make them as expensive as the commercial complementary foods.Engagement in work outside the home was a constraint on the length of time the mother spent on child feeding and care. Engagement in work by mothers may lead to reduced feeding frequency hence low nutrient intake. One study (Hotz and Gibson, 2005) attributed increased energy intake from complementary foods to more frequent feeding. However, a study from India (Singh et al, 2005) found no additional benefit of increasing complementary feeding frequency on likely due to lower breast milk intake. A food that is considered good on the basis of its nutritional value and freshness and that which can be readily prepared has potential market among Chilenje mothers.

The wide support for breastfeeding is supported by the observation that only 2 of the mothers introduced complementary foods due to reported perception of lack of enough breast milk. The median age for the introduction of complementary foods was 6 months as recommended (WHO, 2002), but some mothers introduced these foods too early at 2 months and some too late at 12 months. The acknowledgement of maternal HIV status as a determining factor for the early introduction of complementary foods supports previous observations that knowledge that HIV can be transmitted through breastfeeding might result in decreased levels of breastfeeding (Omari et al. 2003). This may reflect a response to current recommendations for abrupt cessation of breastfeeding for HIV-infected mothers in cases where affordable, feasible, acceptable, sustainable and safe replacement feeding is available (WHO, 2002). Other work from the same population (Chisenga et al. 2005) showed that HIV-infected primiparous women stop exclusively breastfeeding earlier than other women in Chilenje, suggesting that there is confusion about what is the best feeding practice for HIV-infected women in resource poor settings where the strict criteria for replacement feeding may not be easily met. A recent review (Dewey et al. 2004) further highlighted difficulties in constructing a nutritionally adequate diet for the non-breastfed child in low income settings.

The observation that mothers/caretakers encouraged the child to eat only when the child was not eating well is contrary to the currently recommended responsive feeding (Lutter, 2003) that entails feeding with positive verbal encouragement, without physical or verbal coercion (Pelto et al. 2003) and increasing the feeding frequency in response to the child's demand for food (WHO, 2003b). A recent

study from Malawi (Hotz and Gibson, 2005) demonstrated that encouraging children to eat by mother had positive impact on dietary adequacy. The fact that most feeding sessions took place in the living room could result in interruption of feeding by visitors and entertainment media such as television. The significance of a feeding environment free of distraction for adequate nutrient intake has been underscored (Pelto et al. 2003).

The observed daily intake of food solids is within the range (< 25 g to > 250 g) reported earlier for developing countries (Lutter and Dewey, 2003). The observed total intake of absorbable iron and calcium fell short of recommended values (Lynch and Stoltzfus, 2003, Lutter and Dewey, 2003). The observed intake of iron in the first phase of this study is consistent with recent observations from Tanzania (Mamiro et al, 2005) that complementary foods provided 15%, 20% and 27% of iron requirements for infants 6-8, 9-11 months and young children 12-23 months, respectively. The current results also support recent findings from South Africa (Faber, 2005) that complementary foods were inadequate in iron, zinc and calcium.

The observed high intakes of vitamin A can be attributed to the fact that in Zambia sugar is fortified with vitamin A at a minimum rate of 10 mg/kg (Serlemitsos and Fusco, 2001). The mean daily food solids intake observed in the randomized controlled trial were 92.7 sd 52 (range 10.7-245) g, 81.7 sd 49 (range, 15-278) g and 86.6 sd 47 (range 10.5- 259) g for CBM, CBMA and control groups, respectively. From Figure 5-5, it is estimated that infants received about 55% of their total daily nutrient intake from porridges. From 24h-recall data,

sugar formed 20% of porridge dry recipe. From Table 5-22 infants in CBM, CBMA and control groups received 278 μg, 194 μg and 153 μg vitamin A, respectively from complementary foods. Thus it is estimated that vitamin A intake from sugar in the three groups accounted for 36%, 46% and 63% of the total vitamin A intake from complementary foods for infants in CBM, CBMA and control groups, respectively. The differences between groups is attributable to the fact that infants in CBM and CBMA groups received fortified study blends, hence the lower proportion of vitamin A attributable to sugar intake in the two groups. This finding is consistent with the results of evaluation studies on the effect of sugar fortification on vitamin A status in Zambia (Serlemitsos and Fusco, 2001). Since vitamin A is classified as one of the problem micronutrients in developing countries (Dewey and Brown, 2003), the Zambian model of fortifying sugar with vitamin A may be used in other countries. However, sugar may not be affordable to most households in poor settings and may thus not be the best vehicle for vitamin A. Micronutrient capsules or sprinkles (Dewey and Brown, 2003) may be more appropriate for poor settings. However, the cost of micronutrient capsules and sprinkles and the risk of micronutrient toxicity due to overdosing of some nutrients such as iron and vitamin A must be considered.

That the intakes of iron, calcium and zinc were found to be inadequate in the current study further augments previous observations that these two micronutrients are among 'problem nutrients' in developing countries (Dewey and Brown, 2003) even in cases where strate.g.ies to improve their bioavailability are employed (Gibson et al. 1998). A recent double-blind, randomized placebo-controlled study from Tanzania (Mamiro et al. 2004) showed that improved iron

solubility and energy density of a complementary food processed using germinated and autoclaved finger millet-kidney beans, roasted peanuts and mango puree had no effect on growth, haemoglobin concentration and iron status of children 6-12 months old. The workers concluded that the slight improvement in iron solubility as a result of processing was not sufficient to offset the low iron content of the complementary food. A study from Bangladesh reported inadequate intake of vitamins and minerals among breast-fed infants 6-12 months old and attributed this to low micronutrient density of complementary foods (Kimmons et al. 2005).

The findings of the current study strongly support previous recommendations that multi-micronutrient fortification of complementary foods is required if the needs for 'problem nutrients' like iron, calcium and zinc are to be met, especially for the non-breastfed infants after the first 6 months of life.

6.3.2 Development, acceptability and costing of complementary food

The results show that it is feasible to develop affordable, acceptable, and shelf-stable and nutritionally complete complementary food based on locally available cereals and le.g.umes. The observation that addition of α-amylase resulted in better porridge taste and thickness can be explained by the fact that the enzyme breaks down starch into maltose and dextrin units thereby resulting in enhanced sweetness and reduced swelling capacity. Additional oil level due to higher groundnuts content of blend 1 resulted in higher viscosity, especially without α-amylase and explains the observation that this blend had higher scores (lower

acceptability) for thickness compared to the other two. The dark colour observed for the extrusion cooked α-amylase treated fortified blend that led to complaints by mothers may be attributed to several factors which have acted synergistically. Firstly, the variety of common beans used (*kabulangeti*) has a dark skin and upon cooking results in very dark grey broth.

Secondly, Maillard reaction products, such as furans and and sulfur-containing compounds, may result from high temperature, low-moisture extrusion cooking (Parker et al, 2000), though these volatile products may also enhance flavour in thermally processed products (el-Kayati et al, 1998). Interactions between reducing sugars (e.g. glucose) and amino acids during Maillard reactions lead to decreased protein biovailability, especially that of lysine (Yeung et al, 2006), an essential amino acid. Thirdly, the vitamin-mineral premix used for fortification in this study had grey colour and may have exacerbated colour deterioration. A recent study from Mexico (Rosado et al, 2005) found similarly low sensory scores for color in a study designed to assess the effect of fortification on maize flour stability and acceptability. However, there were no observable differences in colour between fortified and non-fortified porridge blends in the current study. Overall the observation that both roasted and extrusion cooked porridges were generally accepted agrees with previous observations that mothers often find improved foods processed from locally available staples attractive (Mensa-Wilmot et al, 2001).

The observation that mothers liked α-amylase-treated porridge due to its sweetness is consistent with findings from previous studies (Darling et al, 1995;

Vieu et al, 2001). The Codex Standard (Codex Alimentarius, 1981) for processed cereal-based foods for infants and children states that reconstituted dry cereal should be suitable for spoon feeding of infants and children. Previous studies have reported that mothers preferred semi-liquid, easily spoon-able porridges with various viscosity ranges including 2000-6000 cp (Gopaldas et al, 1988) and 1000-3000 cp (Mosha and Svanberg, 1993). The high viscosities of the traditional porridges can limit the amount consumed by infants and young children due to the high bulk.

In a previous study in South Africa (den Besten et al, 1998) amylase was applied at 0.05% at 23% flour in porridge. The diets in the South African study had dry skimmed milk and the viscosities were lower than the maize-le.g.ume blend in the present study. A recent study from India (Chakravarthi and Kapoor, 2003) used much higher α-amylase application rate (0.1% w/w) to achieve viscosity range 1000-3000 cp in wheat-green gram and sorghum-green gram blends prepared at 35% slurry concentration. The energy density (90 kcal/100 ml) for traditionally prepared porridges in the current study compares well with the 117 kcal/100 ml for multi-mixes reported previously (Hayes et al, 1994) for high-density population areas of Lusaka. Multi-mixes were described as foods incorporating staples, protein food supplements, energy supplements, and ve.g.etables and fruits.

The observation that extrusion cooking resulted in more viscosity decrement upon addition of α-amylase compared to roasting may be explained by the de.g.ree to which each cooking technique modifies starch. Extrusion cooking has been

suggested as one way of reducing dietary bulk in weaning foods (Iwe, 1998; Treche and Mbome, 1999) possibly due to thermal hydrolysis of starch (Moraru and Kokini, 2003). Addition of 0.04% (w/w) α-amylase results in a 1.4% increment in unit cost of the developed blend and is an affordable method to increase porridge slurry concentration. The total cost (per kg^{-1}) was less than US$4, the average price of commercially available complementary foods.

The observed water activity, microbiological count and acceptability scores for extrusion cooked amylase-treated fortitied blend after 6 months of storage are consistent with recent data from Mexico (Rosado et al, 2005) for fortified maize flour stored for 3 months at 22 °C. However, the current study is different from the Mexican study in that Chilenje Baby Mix was prepared from whole maize in contrast to the degermed maize used in Mexico. Degermed maize is expected to have longer shelf stability due to low lipid content. However, the low blend water activity observed in the current study was expected to result longer stability in storage despite the use of whole maize.

The observation that micronutrient premix and associated freight and cost accounted for almost 60% of the total product cost may be attributed to the small scale of blend production and the importation of vitamins and mineral premixes. This may be avoided in future by larger scale production. Secondly, the feasibility of local micronutient premix manufacture should be assessed. The scale up of food fortification activities in developing countries may be constrained by limited capacity (WFP, 2006b). Local micronutrient premix production may be

hampered by lack of technical capacity in handling and storage of micronutrient premixes (WFP, 2006b) hence safety may not be guaranteed.

6.3.2.1 Implications for future blend scale-up and marketing

Focus group discussion results indicated mistrust for commercially available complementary foods by all participants (mothers, fathers and nurses). However, the study blends were well accepted by mothers. Nutrient intake data also show that mothers with low breast milk output or those who are not breastfeeding opt to buy commercial complementary foods. The most feasible strate.g.y for the marketing of the developed blend is to distribute it through the health provision system and non-governmental organizations (NGOs) by emphasizing its benefits on micronutrient status. A good example is the promotion of vitamin A-fortified sugar by the Ministry of Health in Zambia.

6.3.2.2 Aflatoxins

Infant growth in the study population may also be affected by high aflatoxin exposure due to the wide use of groundnuts for complementary food preparation. Consumption of groundnut and maize-based complementary foods has been associated with growth faltering (E.g.al et al, 2005). Although both aflatoxin exposure and impaired gut permeability are associated with growth faltering, (Gong et al, 2002; Gong et al, 2004; Campbell et al, 2003) these factors were not included in the study design due to budgetary and time constraints. High levels of aflatoxins occurring in human breast milk have been previously reported (Coulter et al, 1984; el-Nezami et al, 1995; Abdulrazzaq et al, 2003). Although efforts

were made to ensure that the processed blend had very low aflatoxin levels, aflatoxin levels in traditional complementary foods were not assessed.

Groundnuts are widely consumed in most African countries (Bankole and Adebanjo, 2003) and used as a main ingredient the preparation of complementary foods, especially in the Central and South African re.g.ions. Groundnuts and maize are among foodstuffs that are most susceptible to aflatoxin contamination (Kaaya and Warren, 2005). The results show that the physical removal of groundnut kernels highly contaminated with aflatoxins prior to complementary food processing resulted in undetectable aflatoxin levels in the processed blend, but resulted in higher groundnut cost as charged in the market. The results also show that the processed blend had both low moisture content and water activity indicating that it was inhibitory to mould growth and aflatoxin production in storage. The observed a_w of 0.5 (SD 0.01) was lower than the minimum a_w (0.7) for mould growth and aflatoxin production (Moss, 1991).

Although aflatoxin reductions observed in the present study are largely attributable to the removal of highly contaminated groundnut kernels, extrusion cooking may also result in reduced aflatoxin levels. Reductions of aflatoxin levels as a result of extrusion cooking of up to 95% have been reported (Castells et al, 2005). These results support previous studies (Fandohan et al, 2005; Galvez et al, 2003; Drusch and Ragab, 2003) that demonstrated that sorting can be used alone or in combination with other strate.g.ies to produce aflatoxin-free foods. Recent work from Guinea showed post-harvest intervention strate.g.ies including sorting resulted in reduced aflatoxin biomarkers in blood (Turner et al, 2005) indicating that it is possible, as in our study, to prepare complementary foods with

undetectable afaltoxin levels for children in Africa. The reduction of the risk of growth faltering and immune suppression, especially among infants and young children born to HIV-positive mothers is a major health benefit.

6.3.3 Randomized controlled trial

The results show that consumption of fortified maize-beans-bambaranuts-groundnuts blends, re.g.ardless of amylase treatment, resulted in improvement in length and hemoglobin whereas weight gain was not affected. Infants in both intervention groups gained more fat. Breast milk intake did not differ between any of the groups and it is apparent that traditional complementary foods were displaced by the provision of higher quality complementary foods.

6.3.3.1 Infant growth and haemoglobin concentration

The observation that the provision of the study foods had little effect on growth over 3 months may be explained by the fact that energy and protein intake were not limiting in the study population. Secondly, the intervention duration may not have been long enough for larger impact on growth. Any benefit of the blends on growth could possibly have been confounded by the fact that some infants may have been HIV-exposed. HIV prevalence in the study population is high and is estimated to be 30% (NDHS, Zambia, 2003). Stunting marked by decreased lean body mass is common in HIV-infected infants (Arpadi, 2000). HIV-infected infants are usually shorter and lighter than uninfected children at birth and this trend remains several months after birth in both sexes (Moye et al, 1996). HIV infection is associated with higher prevalence of underweight in Southern Africa

(Mason et al, 2005). HIV infection could also result in poor intestinal permeability (Rollins et al, 2001) which may in turn lead to malabsorption of nutrients.

Parasitic infection such as *Gardia lamblia* may cause impaired gut permeability (Goto et al, 2002) and is associated with low height-for-age in children under 2 years of age and low weight-for-age in older children (Oberhelman et al, 1998).

The observation that WAZ, HAZ and WHZ declined rapidly to below the reference line can be explained in several ways. Firstly, the fact that predominantly or partially breastfed infants grow faster than the NCHS reference until 6 months of life, after which their growth is slower than the NCHS reference (Victora et al, 1998) is now a well known phenomenon. A ne.g.ative association between the de.g.ree of breastfeeding at 2.5 months and weight gain in in the first 6 months months of life has been observed (Ekelund et al, 2006). Longer periods of breastfeeding as observed in the current study may be protective against the risk of overweight and obesity in later life (Gillman et al, 2001) as a result of lower weight gain in infancy (Ekelund et al, 2006). Growth faltering despite continuation of breastfeeding combined with the provision of adequate complementary food in the first of year of life may also be attributed to prenatal factors (Dewey, 1998). It has been demonstrated that neonatal weight and length, reflectors of prenatal environment, strongly predict infant nutritional status at 6-7 months of age (Schmidt et al, 2002).

The current study shows that both intervention and control infants were receiving more than 100% of the recommendations (Lutter and Dewey, 2003) for energy intake. Macronutrients were therefore not a limiting factor and any differences

observed in growth and haemoglobin concentration can be attributed to multi-micronutrient fortification of the study blends.

The results of the current study are consistent with work from Congo (Moursi et al, 2003) that found improvement in length between 24-31 weeks in infants given multi-micronutrient fortified maize-soya blend. However, no significant effect of α-amylase addition on length was observed in the current study in contrast to the Congolese study. The main differences between the current and the Congolese study include the fact that in the latter case complementary foods were introduced at a younger age (13 weeks), the initial stunting rate (15.5%) was higher than that observed at 6 months in the current study and meal frequencies were low with 25%, 54% and 21% of infants receiving one meal, two meals and more than two meals per day, respectively. Although an increased energy intake from amylase-treated complementary gruel was observed in the Congolese study, there was no impact on total daily energy intake. Energy intake may have been limiting in the Congolese infants hence the positive effect of nutritional intervention. Previous studies assessing the effect of α-amylase on energy density of complementary foods and infant growth (Table 2-2, Section: 2.2.2.3.1) have demonstrated a positive effect on growth and energy intake in severely malnourished children with acute diarrhea. A study in Jamaica designed to assess the effects of weaning-food viscosity and energy density on consumption and energy intake in 15 non-breast-fed children aged 7-15 mo (Stephenson et al, 1994) found that amylase treatment of a thick energy-dense porridge did not increase intake further.

The observed improvement in length by giving fortified complementary foods for only 3 months in the current study is contrary to results from published studies (Table 2-4) that showed no improvement in growth with the provision of fortified blends in infants (Oelofse et al, 2003; de Almeida et al, 2003). A Ghanaian study (Lartey et al, 1999) found intervention effect on weight-for-age and length-for-age between 9 and 12 months, but not between 6 and 9 months. Multi-micronutrient supplementation studies (Hop et al, 2005; Lopez de Romana et al, 2005; Smuts et al, 2005; Untoro et al, 2005) in infants have also reported no benefit on growth. Benefits of a fortified beverage on weight, height and BMI were reported in older children (Abrams et al, 2003).

A recent study from South Africa (Faber et al, 2005) found no effect on weight, length or growth Z-scores of giving infants fortified maize meal porridge for 6 months. One limitation in the South African study was that the level of zinc fortification was lower than the current recommendations for infants 6-12 months old (Lutter and Dewey, 2003). The new zinc fortification levels were used in the present study. Although the effect on infant of food fortification with zinc alone has not been assessed, supplementation of zinc alone has been shown to improve growth in stunted infants (Rivera et al, 1998; Umeta et al, 2000). It is likely that infants in the current study were zinc deficient at baseline. The effect of fortified complementary foods on zinc status warrants further investigation.

Baseline body composition data (skinfolds and circumferences) were not available for the control group. However, comparisons of fat mass at 9 months was based on the assumption of no significant differences in basleine body composition

indices since there were no significant differences among the three groups in birth weight and weight at 6 months. The observation in the current study that the consumption of the porridge blends resulted in significantly greater percent fat mass and skinfold thickness (biceps, triceps, subscapular and suprailiac) without significant differences in weight gain is unexpected. However, it is possible that the presence of zinc in the fortification premix resulted in fat increment. A zinc supplementation study in Guatemala (Cavan et al, 1993) showed significant increment in triceps skinfold in children. This result may be comparable to a past study in Britain (Wells et al, 2002) that found that contemporary children have greater fatness than the reference child. Although the workers attributed this observation to the inadequacy to measure fatness, it could also be due to change in dietary intake characterized by more energy-dense foods. Rapid weight gain in infancy is a risk factor for later obesity (Wells et al, 2006; Ekelund et al, 2005) and insulin resistance (Singhal et al, 2003). However, the effect of fatness in infancy on later obesity has not been adequately studied. This is important due to the nutrition transition occurring in developing countries with higher availability of energy-dense complementary foods (Uauy et al, 2001).

The effect of study blends on body composition of infants is contrary to findings from Ghana (Lartey, 1999) where all body composition indices except MUAC, head circumference and mid arm muscle area deteriorated over 6 months of intervention. Mean measurement and changes in body composition indices, except head circumference, between 6 and 9 months in the current study were higher than those reported from Ghana.

The observed signigificant improvement in haemoglobin concentration with the consumption of the study blends supports was expected and is attributable to the fact that the blends were adequately fortified with micronutrients including iron. Although this study was not designed to measure micronutrient status, it is likely consumption of the blends also resulted in improved micronutrient status. This is indicated by the trend towards improved length which may be due to the zinc content of the blends.

The observation in the current study of improved haemoglobin concentration levels (mean 5.5 g/L) with the provision of the blends agrees with a recent finding from South Africa (Faber et al, 2005) that fortified maize-meal porridge resulted in 9 g/L differences in haemoglobin concentration. The differences in effect levels between the two studies may be explained by the fact that the in South African study the length of follow up was 6 months compared to only 3 months for current study. Additionally, there was more intense follow up and encouragement to consume the study food in the South African study than was done in the current study. A study from Chile (Torrejon et al, 2004) reported favorable effects of fortified powdered cow's milk on iron status of young children until 18 months. Two recent multi-centre studies (Hop et al, 2005; Lopez de Romana et al, 2005; Smuts et al, 2005; Untoro et al, 2005) assessing the effect of multiple micronutrient supplementation on infant growth and micronutrient status demonstrated significantly favorable effects of daily multi-micronutrient supplementation on infants' iron status.

That the consumption of porridge blends in the current study resulted in significantly higher haemoglobin concentration than in control children after 3 months of follow up is contrary to findings from similar studies (Table 2-4) using cereal-le.g.ume complementary blends from Ghana and South Africa (Lartey et al, 1999; Oelofse et al, 2001) that showed no improvement in haemoglobin concentration after 6 months of followup. In contrast to the current study, the Ghanaian study was based in a rural area with high malaria prevalence. Malaria infection has been associated with low haemoglobin concentrations during infancy (Ndyomugyenyi and Magnussen, 2000). Secondly, although the complementary foods in both studies were centrally processed, it is possible that the Ghanaian food, which was used as a general distribution food aid, may have had lower micronutrient levels compared to the blends used in the current study that were fortified with higher levels of micronutrients specific to infants 6-9 months age group. Recent work from Haiti (Ruel et al, 2004) showed that although donated complementary foods were adequate in meeting infants' energy needs, these foods could not meet infant iron and zinc needs.

The current work is among the first few studies to report evidence of improved haemoglobin concentration and moderate effect on length of a centrally processed multi-micronutrient fortified complementary food predominantly breastfed, healthy and relatively advantaged urban infants within only 3 months of intervention.

6.3.3.1.1 Breast milk intake

The observation that the three groups had no significant differences in breast milk intake is not surprising since it was expected that the provision of the study food would only result in displacement of traditionally used complementary foods. The differences in breast milk intake between the control group and CBM and CBMA were 39 g/d and 18 g/d. This finding is of significance since it shows that high quality complementary foods can be incorporated into infants' diet without significant reduction in breast milk intake. Similar work from Congo (Moursi et al, 2003) showed that the consumption of α-amylase-treated fortified gruels did not affect breast milk intake of infants at 6 months of age. Some recently published work (Bajaj et al, 2003; Haisma et al, 2003) has, however, shown a tendency for breast milk displacement by complementary foods. A report from India (Bajaj et al, 2005) showed that infants 6-10 months old given a complementary food of higher density had lower breast milk intake with a mean difference of 121 g/d. The differences in breast milk intake between the current study and published data may be explained by age differences, complementary feeding practices and breast milk measurement technique and socio-economic factors. Table 6-1 presents existing studies on breast milk intake in developing countries.

WHO currently recommends exclusive breastfeeding for the first 6 months of life (WHO, 2002) before introduction of complementary foods. It is expected that at 9 months all infants receive complementary foods, hence lower breast milk intake. The low breast milk intakes reported from Congo and India may be due to differences in complementary feeding practices. Mothers in the Congo study were

reported to have introduced complemetary foods by 2 months of age. This is the first study to report breast milk measurement by deuterium-dose-to-the-mother technique in 9-month old infants in a developing country. Breast milk measurement technique may also cause differences among different studies. While deuterium-dose-to-the-mother technique is accurate and non-invasive, test-weighing is inaccurate (Coward, 1984) and may interrupt customary feeding patterns and normal mother-infant interaction (Coward et al, 1979).

Underestimation of breast milk intake by test-weighing occurs due to insensible water loss during breastfeeding (Wells and Davies, 1995). The low breast milk intake observed in the Kenyan study was attributed to harsh living conditions (Ettyang et al, 2005) among pastoral communities. Improved maternal nutritional status and higher socioeconomic status may result in improved breast milk output (Gonzalez-Cossio et al, 1998).

The observation in the current study that there were no significant differences in non-milk water intake agrees with our 24-h recall results showing that there were no differences in energy and macronutrient intake. Non-milk water intake is an estimate of energy and macronutrient intake (Haisma et al, 2005). The mean non-milk water intake observed in the current study, 450 (95% CI: 349, 551) g/day is higher than the reported mean level, 395 (95% CI: 225, 566) g/day reported from Brazil for partially breastfed infants at 4 months of age (Haisma et al, 2005).

Table 6-1 Studies reporting breast milk intake measurement in developing countries

Study by breast milk measurement method	Country	Age (months)	Sample size	Amount of breast milk (g/d)	Comments
Deuterium-dose-to-the-mother dilution					
Haisma et al, 2003	Brazil	4	70	746	
Ettyang et al, 2005	Kenya	2-4	10	552	
Villalpando et al, 1992	Mexico	4	30	885	Minimal infant food supplementation. No effect of current diet on milk production
		6		869	
Rosetta et al, 2005	Bangladesh	12	30	710	Tea workers had lower milk output than non-tea workers (672 vs 749 g/d). Doubly labeled water method
This study	Zambia	9	53	639	
Test-weighing					
Moursi et al, 2003	Congo	6	75	388	
Bajaj et al, 2005	India	6-10	20	447	
Gonzalez-Cossio et al, 1998	Guatemala	6		764	Mothers received food supplementation 5-25 weeks post-partum
Butte et al, 1984	Mexico	1		751	Designed to assess effect of maternal diet on breast milk production
		2	45 (total for	725	
		3	infants 1-4	723	
		4	months)	740	

6.3.3.2 Nutrient intake

6.3.3.2.1 Nutrient intake from complementary foods

The results show that there were no significant differences in energy, fat, protein, fibre, niacin, pyridoxine and phyate intake from complementary foods, however the control group had significantly lower intakes of calcium, iron, zinc, vitamin A, vitamin C, thiamine and riboflavin, and greater phytate:zinc and phytate:iron molar ratios compared to CBM and CBMA. The daily energy intake from complementary foods in all groups was more than 100% of the RDA for energy intake from complementary foods for infants 6-9 months old. The observation that study complementary foods resulted in higher micronutrient intake was expected. The use of Chilenje Baby Mix in both CBM and CBMA resulted in partial displacement of commercial complementary foods but not of breast milk. We did not expect this to affect the results ne.g.atively since Chilenje Baby Mix met the requirements for infant nutrient needs.

The observations that energy intake from complementary foods was adequate in all groups, but that the intakes of iron, zinc and calcium and other micronutrients were low in the control group support results from similar studies (Moursi et al, 2003; Ruel et al, 2004; Faber et al, 2005).

6.3.3.2.2 Overall nutrient intake

The results show that there was adequate energy, vitamin A, vitamin C, thiamine, riboflavin, niacine and pyridoxine intake. However, there was suboptimal intake of calcium, iron and zinc without intervention. Since there were no differences in breast milk intake among the three groups, the improvement in calcium, iron and

zinc in CBM and CBMA can only be attributed to intake from the study complementary foods. This observation supports work from Congo (Moursi et al, 2003) that found increased iron and zinc intake among infants given α-amylase-treated, multi-micronutrientfortified maize-soya blend.

6.4 Application of study results

6.4.1 Already accomplished

This study was carried out as a pilot phase of a larger trial namely, Chilenje Infant Growth Nutrition and Infection Study (CIGNIS) using the complementary blend in the same clinic. CIGNIS is funded by the Bill and Melinda Gates Foundation. The results of the current study were useful in the study design for CIGNIS in several ways. Firstly, changes in the variety of beans (*Phaseolus vulgaris*) from dark-skinned *kabulangeti* to *white and yellow* and white maize to yellow maize were recommended in order to improve blend colour and general acceptance.

Originally CIGNIS was designed to assess in a larger sample of infants the impact of improved complementary food energy density by addition through α-amylase. However, the results of the current study showed that the intake of micronutrients, and not energy was limiting in the study population. CIGNIS was redesigned to assess the long-term (12 months) impact of the blends on micronutient status of a larger sample of (850) children. Apart from growth, child development outcomes will be measured alongside micronutrient (vitamin A, zinc, copper, iron) status, immune function, and mental and motor development. These additional measurements may help bridge the limitations identified in the current study.

In addition, experience gained in the establishment of the isotope method for measuring breast milk intake will be replicated in Lusaka to measure breast milk output of HIV-infected and uninfected women earlier in lactation in a planned study.

6.4.2 Future applications

The current study shows that breastfeeding status affected complementary feeding patterns. In circumstances of high HIV-prevalence such the study population, mothers may stop breastfeeding prematurely in order to avoid the risk of HIV transmission through breastfeeding. This study shows that although non-breastfeeding mothers bought commercial replacement foods, they may not afford adequate amounts to meet entire infants' nutritional requirements, especially micronutrients. Based on this knowledge a two-phased study has been designed to assess by stable isotope techniques the impact HIV infection in early infancy on breast milk intake and the impact a cheap fortified maize-bean blend similar to the one used in this study on growth and body composition of HIV-ne.g.ative infants of HIV-positive mothers in a Kenyan slum. The first phase of the proposed work has already received funding from the International Atomic Energy Agency for 3-year term and will measure breast milk intake at 5 months of age of HIV-infected and uninfected infants born to HIV-infected mothers. The second phase of the proposed work will comparatively assess the impact of a 6 month intervention from 6-12 months of Unimix (Corn Soy Blend) and maize-bean-groundnut blend on growth, body composition, zinc and iron status and aflatoxin exposure in HIV-ne.g.ative infants of HIV-positive mothers.

6.4.3 Policy implications

The current recommendation for infant and young child feeding (WHO, 2002) is that infants be exclusively breastfed for the first six months of life and thereafter should receive appropriate complementary foods with continued breastfeeding for up to two years or beyond (IWGFNC, 2004). However, the feeding of infants of HIV-infected mothers or infants whose mothers are dead presents a special challenge (IWGFNC, 2004; Dewey et al, 2004). In order to reduce the risk of mother-to-child transmission of HIV mothers are advised to avoid breastfeeding from birth if they have access to acceptable, feasible, affordable, sustainable and safe replacement feeding (WHO, 2002). In resource poor settings are advised to exclusively breastfeed for the first six months then abruptly stop breastfeeding (WHO, 2002). Recent evidence shows that some mothers who know that they HIV-infected still continue to breastfeed even in cases where there is counseling on infant feeding options (Chisenga et al, 2005). This has been attributed to lack of openness among partners, fear of stigma in the community (Chisenga et al, 2005), lack of knowledge (Chisenga et al, 2005; Kiamba et al, 2005) and lack of money (Kiamba et al, 2005; Petrie et al, 2005) to buy replacement formulas.

This subsection discusses the feasibility of using Chilenje Baby Mix in complementary of breastfed infants after and in replacement feeding of infants who are not breastfed due to known maternal HIV-infection. Since Chilenje Baby Mix was designed for feeding of infants after 6 - 9 months, the discussion is limited to this age group.

6.4.3.1 Use of Chilenje Baby Mix in complementary feeding of breastfed infants

The results of this study show that Chilenje Baby Mix mix can be used in combination with other traditional complementary foods and family foods with continued breastfeeding to meet entire energy requirements. The energy density of Chilenje Baby Mix [approximately 25 g dry flour in 1 cup of water (250 ml] is 0.68 kcal/g. It recommended (Dewey and Brown) a coplementary food with an energy density of 0.71 kcal/g with medium breastmilk intake be fed 2 times a day to attain the level of energy needed fron infants 6-8 months old. To cover the age upto 11 months of age, this study proposes that Chilenje Baby Mix be fed 3 times a day. Assuming that infants consume 35 g of dry Chilenje Baby Mix a day, they will obtain 145 kcal from this mix. At the observed average breast milk intake (639 g/d) infants will obtain 428 kcal from breast milk. The current estimates for infants' energy requirements are 615 kcal/d and 686 kcal or infants 6-8 months and 9-11 months (Dewey and Brown, 2003), respectively. The gap in energy intake (52 kcal/d and 123 kcal/day for infants 6-8 months and 9-11 months, respectively) can be met from traditional complementary foods and family dishes. The other foods should include about 200 ml/day milk (cow, goat, sheep, buffalo, camel) and other animal-source foods, vitamin A and vitamin C rich foods (IWGFNC, 2004). Milk can be added during the preparation of Chilenje Baby Mix.

6.4.3.2 Use of Chilenje Baby Mix in replacement feeding of non-breastfed infants.

A strate.g.y to inte.g.rate food aid with programmes for Prevention of Mother to Child Transmission (PMTCT) of HIV is currently being developed by the World Food Programme (WFP) to provide infants and young children of HIV-postive mother in resource poor settings with replacement foods such as corn-soya blend or an equivalent after the cessation of breastfeeding (IWGFNC, 2004). Chilenje Baby Mix may one of the fortified blends that may be considered for the provision of micronutrients to nonbreastfed infants. For the feeding of nonbreastfed infants 6-11 months old, this study proposes 5 servings of Chilenje Baby Mix (prepared as in the preceding section 6.4.3.1) per day every 3-4 hours. Based on an approximate 50 g/day of CBM, infants will obtain 207 kcal/day from this source. Based on recommendations that nonbreastfed infants at least 400 – 600 extra fluids in addition to the 200-700 ml of water from milk and other foods (IWGFNC, 2004) this study proposes that milk be next major source of nutrients. Infants should be given at least 3 glasses (approximately 600 ml) of milk to provide about 366 kcal. Such milk should be heat treated, for example by bringing to boil by direct heating in a sauce pan or indirectly by putting milk in a cup placed in a water container that is heated over a flame, to avoid any potential occult blood loss (IWGFNC, 2004). Occult blood loss may be due to pathogens that are present in milk. Heat treatment results in the destruction of milk pathogens, hence avoidance of blood loss (Dewey et al, 2004). The gap in energy intake (42 kcal/d and 113 kcal/d for infants 6-8 months and 9-11 months, respectively) can be met from traditional complementary foods and family dishes.

7 Conclusions and recommendations

7.1 Conclusions

The benefits of the porridge blends for infant growth were modest; however, there is greater evidence for benefits on haemoglobin concentration and, likely, micronutrient status which could lead to improved child health. Measurement of breast milk intake using the sensitive and accurate stable isotope method indicated that provision of the complementary foods did not have the adverse side effect of displacing breast milk. The industrially processed fortified complementary foods based on locally available and widely used le.g.umes and cereals are about half the cost of nutritionally comparable commercial infant foods and could be used to improve growth and haemoglobin concentration among infants in middle income communities in developing countries without adverse effect on breast milk intake. Further work on the effect of improved complementary foods on growth, body composition and micronutrient status of non-breastfed infants is recommended.

7.2 Recommendations

The use of locally available cereals and le.g.umes to process improved complementary foods should be further encouraged to enhance the acceptability and to complementary food cost.

This study adds to existing evidence that although the addition of α-amylase to complementary foods is a simple and cheap method of improving the energy density, it does not result in increased nutrient intake or improved growth in free-

living infants whose energy intake is apparently adequate. However, the use α-amylase to improve nutrient intake from complementary foods among severely malnourished infants should be promoted. In cases where mothers are given α-amylase-treated complementary foods to prepare at home there should simple and clear instructions on flour-water mixing to ensure the desired porridge energy density is achieved.

The findings of this study indicate that non-breastfed infants may not receive enough nutrient levels from replacement foods to allow for adequate growth and haemoglobin concentration. There is currently no adequate data on the benefits of locally processed or commercial fortified cereal-le.g.ume-based replacement foods on growth and nutritional status of non-breastfed infants. Work in this area is urgently needed.

From these findings, there is no suggestion that the provision of improved complementary foods in addition to the usual diet leads to displacement of breast milk in areas where breastfeeding is widely supported.

Based on the results of this study further research is recommended in the following areas:

1. Assessment of the marketability of industrailly processed fortified complementary foods based on locally available cereals and le.g.umes in both urban and rural settings.

2. What is responsible for rapid decline in weight-for-age in infants aged 6-9 months even in the presence of nutritional intervention with fortified complementary foods without breast milk displacement? This question needs to be addressed with focus on 1) HIV-infection or exposure during infancy; 2) high exposure to aflatoxins from traditional complementary foods and; 3) parasitic infections that may compromise gut permeability.

3. What is the impact of fortified complementary/replacement foods (such as Chilenje Baby Mix) on micronutrient status (e.g.. iron, zinc, vitamin A) of infants.

4. Can improved foods such as Chilenje Baby Mix be used to adequately as replacement foods to meet nutritional needs of infants after 6 months of age in circumstances where mothers choose to cease breastfeeding in order to avoid the risk of HIV transmission through breast milk?

5. What is the effect of maternal HIV-infection on infants' breast milk intake in early infancy?

6. There is need to assess the impact of nutritional intervention infant body composition to acertain what comprises weight gain (lean mass or fat mass?). To do this there is need to use accurate body composition assessment techniques such as stable isotopes (doubly labeled water, ^{18}O or deuterium-dose-to-the-infant technique.

7. What is the effect of short-term nutritional intervention with fortified complementary/replacement foods between 6 and 12 months of age on later development of adult diseases such obesity, diabetes or insulin resistance?

8. Assessment of the cost of traditionally used complementary foods relative to commercial alternatives in terms of ingredient cost and processing losses and time spent by mothers in the preparation of these foods.

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9 Appendices

Appendix 9-1 Interview questionnaire for the assessment of complementary feeding practices

QUESTIONNAIRE

A SURVEY OF WEANING PRACTICES IN CHILENJE, LUSAKA

Date of Interview _____

HOUSEHOLD CHARACTERISTICS

Q1. Mother's Name _____

Q2. Mother's Age _____years

Q3. Occupation _____ 1 = Salaried employed
2 = Self-employed
3 = Housewife
4 = Other (specify)
_____ ☐

Q4. Educational Level _____ 1 = Primary (1-7 Grades)
2 = Secondary (8 -12 Grades)
3 = Tertiary colle.g.es
4 = University
5 = Other (specify)
_____ ☐

Q5. Marital Status _____ 1 = Married
2 = Widow
3 = Divorced
4 = Single
5 = Other (specify)
_____ ☐

If married: -
Q6. Husband's Age _____years

Q7. Husband's Occupation _____ 1 = Salaried employed
2 = Self-employed
3 = Unemployed ☐

4 = Other (specify)

_____

Q8. Husband's Educational Level _____ 1= Primary (1-7 years) ☐
2 = Secondary (8 -12 years)
3 = Tertiary colle.g.es/polytechnic
4 = University
5 = Other (specify)

_____

Q9. Total number of persons in the household _____ ☐

Q10. Total number of children in household _____ ☐

Q11. Number of children under 5 years of age _____ ☐

Q12. Age of youngest child _____months

Q13. Sex of youngest child _____ F = Female M = Male ☐

FOOD AVAILABILITY AND HOUSEHOLD FOOD EXPENDITURE

Q14. Which foods do you use to feed your household (*please tick corresponding box and specify which particular food by circling the ones in brackets)*

1 = Cereals (Mealie meal, Wheat flour, Rice, Millet, Sorghum) _____ ☐

2 = Le.g.umes (Soya flour, Beans, Peas, Bambaranut/*Ntoyo/Njama*) ☐

_____

3 = Oil seeds (Groundnuts) ☐

_____

4 = Dairy products (Fresh milk, Sour milk) _____ ☐

5 = Protein foods (Chicken, Beef, Pork, Fish, Kapenta, ☐
E.g.gs)_____

6 = Ve.g.etables (Rape, *Chibwabwa*, Cabbage, *Bondwe,* Carrots, ☐
Tomatoes,
Spinach, *Kalembla*/Sweet potato leaves, Okra, Cassava leaves)

7 = Fruits (Bananas, Avocado, Apple, Pawpaw, Oranges, Lemons) _____ ☐

8 = Tubers/Roots (Irish potatoes, Sweet potatoes, Cassava) _____ ☐

9 = Other (specify) _____

Q15. Which meals did you feed your family yesterday?

MEAL EATEN (TIME)		FOOD EATEN	MAIN INGREDIENTS
1			
2			
3			

Q16. Do you earn any money from any kind of work outside the home?
Y= Yes N = No

Q17. Who decides how much money will be spent on foods for your child?

Q18. Do use the following foods? If yes, what is the source of food? If food is bought, how much money do you spend on the food per month?

Food	**Is Food used Y = Yes, N = No**	**Source of food 1 = Bought 2 = Gift (specify) 3 = Own garden 4 = Other (specify)**	**Quantity of food per month**	**Amount of Kwacha per month**
Mealie meal				
Cassava meal				
Soya flour				
Raw soya seeds				
Groundnuts				
Pounded groundnuts				
Kapenta				
Beans				
Cow peas				
Njama (Bamb. nuts)				
Fresh maize				
E.g.gs				
Fresh milk				
Sour milk				
Pumpkins				
Chibwabwa (pumpkin leaves)				
Bondwe (Amaranth)				
Irish potatoes				
Sweet potatoes				
Cerelac				
Vitaso				
Purity				
Butter				
Cooking oil (salad)				

Oranges
Bananas
Avocado
Pawpaw
Apples
Sugar
Spinarch
Rape
Beef
Fish (not Kapenta)
Chicken
Pork

Q19. Who purchases foods for your child?

1 = Mother
2 = Father
3 = Child' grandmother
4 = Child' aunt
5 = Caretaker
6 = Other (specify) _____ ☐

Q20. Which foods do you use to feed your youngest child? *(please tick corresponding boxes and specify which particular food by circling the ones in brackets).*

1 = Cereals (Mealie meal, Wheat flour, Rice, Millet, Sorghum) _____ ☐

2 = Le.g.umes (Soya, Beans, Peas, Bambaranuts/*Ntoyo/Njama*) _____ ☐

3 = Oil seeds (Groundnuts) _____ ☐

4 = Dairy products (Fresh milk, Sour milk) _____ ☐

5 = Protein foods (Chicken, Beef, Pork, Fish, Kapenta, E.g.gs) ☐
_____

6 = Ve.g.etables (Rape, *Chibwabwa*, Cabbage, *Bondwe,* Carrots, Tomatoes Spinach, *Kalembla*/Sweet potato leaves, Okra, Cassava leaves) ☐

7 = Fruits (Bananas, Avocado, Apple, Paw paw, Oranges, Lemons) _____ ☐

9 = Tubers/Roots (Irish potatoes, Sweet potatoes, Cassava)_____ ☐

10 = Commercial foods (Cerelac, Vitaso, Purity) _____ ☐

11 = Other (specify) _____

INFANT FEEDING AND CARE PRACTICES

A. BREASTFEEDING PRACTICES

Q21. Do you breastfeed your youngest child? Y = Yes N = No

If Yes, proceed to Q22, Q23 and Q24
If No, proceed to Q25, Q26, Q27, Q28, Q29 and Q30

Q22. How many times do you breast feed at day time? _____

Q23. How many times do you breastfeed at night? _____

Q24. At what age of the child do you plan to stop breastfeeding?
_____months

Proceed to Q27

Q25. At what age of the child did you stop breast feeding?
_____months

Q26. Why did you stop breast feeding when the child was at that age?

_____

Q27. How many times daily do you feed your child? _____

Q28. Which foods do you use to feed your child?

_____

Q29. Do you use any other milk to feed your child? Y = Yes N = No

Q30. If Yes, which milk/s do you use to feed your child?

_____
—

B. INFANT CARE AND TIME AVAILABILITY

Q31. How many hours daily do you spend away from home? _____

Q32. Who takes care of your child while you are away?

1 = Father
2 = Child's grandmother
3 = Child's aunt
4 = Child's sibling
5 = Caretaker
6 = Other (specify) _____

Q33. What is normally fed to your child while you are away?

C. COMPLEMENTARY FEEDING PRACTICES

Q34. At what age (in months) did you introduce foods other than breast milk to your child's diet? _____ months.

Q35. Why did you choose this age to introduce other foods?

1 = Advice from nurse
2 = Advice from husband
3 = Child was crying
4 = Lack of enough breast milk
5 = Advice from relatives
6 = Child was old enough
7 = Mother working
8 = Other (specify) _____

Q36. Who decided that it was time to introduce foods other than breast milk to your child's diet at the age you just said?

1 = Mother
2 = Father
3 = Child's grandmother
4 = Child's aunt
5 = Health officer
6 = Other (specify) _____

Q37. At what age (months) did you introduce the following foods into the child's diet?

Food	Age of Introduction	Food	Age of Introduction
Nshima		Ve.g.etable soup	
Mealie meal porridge		Bean soup	
Porridge with soya		Fish	
Porridge with groundnuts		Beef	
Porridge with kapenta		Chicken	
Porridge with pounded beans		Bananas	
Porridge with e.g.gs		Avocado	
Porridge with milk		Oranges	
Sour milk		Rice	
Mashed beans		*Chibwabwa* (Pumpkin leaves)	
Mashed pumpkins		Rape	
Mashed potatoes		*Bondwe* (Amaranth)	
Cerelac			
Vitaso			
Purity			

Q38. What foods are you feeding your child currently?

_____

Q39. Which foods did you feed your child from yesterday to today and at what time?

	TIME CHILD WAS FED	FOOD CHILD WAS FED	QUANTITY OF FOOD	MAIN INGREDIENTS
1				
2				
3				

Q40. Does the child feed herself/himself? Y = Yes N = No

Q41. If No, who Commodities the child?

1 = Mother

2 = Grandmother
3 = Father
4 = Aunt
5 = House help
6 = Child's sibling
7 = Other (specify) _____

Q42. How is food actually offered to the child?
1 = Spoon
2 = Hand
3 = Bottle
4 = Cup
5 = Other (specify) _____

Q43. Does your child receive his/her own serving (plate) of food? Y = Yes N = No

Q44. If No, who shares the same plate with the child?

_____
___

Q45. What factors do you consider when you are choosing food for your child?

1 = Nutritional value
2 = Age of child
3 = Expiry date
4 = Temperature of the food
5 = Other (specify)
_____

Q46. Which foods do you think are very good for your child?

_____

Q47. Why do you say these foods are very good for the child?

_____

Q48. Which foods do you think are not good for your child?

_____

Q49. Why do you say these foods are not good for your child?

_____

FOOD PREPARATION AND HANDLING

Q50. What food items do you use to prepare food for the child?

Q51. What factors do you normally consider important in preparing food for your child?

Q52. What utensils do you normally use to prepare and serve food for your child?

Q53. How much food is normally prepared at one time for your baby? *(let mother quantify e.g. cups, spoons).*

Q54. How do you normally store food that is left after feeding your child?

Q56. Do you use left over food to feed your child? Y = Yes N = No

Q57. If Yes, what do you do before you feed the child with left over food?

Q58. If No, why don't you feed your child with left over food?

Q59. Which fuel (source of fire) do you use to cook food for your child?

Q60. What is the price (Kwacha) of the fuel/s you use to cook for the child?

Q61. How much fuel do you use each time you cook for the child?

Q62. Are there ever shortages of fuel in Chilenje? Y = Yes N = No

Q63. If Yes, how often do fuel shortages occur in Chilenje?

Q64. How many times daily is food cooked for your child?

Q65. Would you like to cook many more times than you do currently? Y=Yes N= No

Q66. If Yes, what makes it difficult for you to cook many more times?

_____

Name of Interviewer _____

Signature _____

Name of Interviewee _____

Signature/Thumb print _____

Appendix 9-2 Questionnaire for acceptability evaluation of complementary food

by mothers

ACCEPTABILITY EVALUATION OF A MAIZE-BEAN BLEND BY MOTHERS OF INFANTS 6– 18 MO AND HEALTH WORKERS IN CHILENJE CLINIC

Date ..... Mother's name .....

Age of youngest child ..... ID: - .....

You are going to taste, smell and see the porridges *(umusunga)* named A, B, C, D, E and F, G, H. Now imagine it is your baby tasting, smelling and seeing these porridges *(umusunga)*. Please choose the face your child will have after tasting, smelling and seeing the porridge.

1 = I like it

2 = I don't know

3 = I don't like it

After you choose your point enter it in the table below. Note: Please rinse your mouth with clean water after tasting each porridge.

	SENSORY ATTRIBUTE SCORE					
PORRIDGE	**Color**	**Smell**	**Taste**	**Thickness**	**Sweetness**	**Liking**
A						
B						
C						
D						
E						
F						
G						
H						

Please answer the following general questions.

1. Which porridge do you like most? .....
2. Would you feed your baby with this porridge? .....
3. How much Kwacha can you pay for a kilo of this porridge? .....
4. What other ingredients would you add to the porridge

.....

5. Which other porridges do you give to your baby? .....

TWATOTELA, LESA AKUPELA!!

Appendix 9-3 Information and consent forms for randomized controlled trial

INFORMATION FOR PARTICIPANTS IN A STUDY TITLED 'EFFECT OF IMPROVED COMPLEMENTARY FOODS ON NUTRITION OF ZAMBIAN INFANTS 6-9 MONTHS OLD'.

It is a worldwide practice to breastfeed babies for sometime after birth. When the baby is about 6 months old, other foods are introduced in addition to breast milk. The type of food chosen and the age at which it is introduced may have an influence on the growth and general health of a baby. The foods used can be ready-made formulas from shops or foods prepared at home. The aim of this study is to understand the amounts of different foods, that is, the food that will be provided by the study project, foods that are traditionally used by mothers to feed children, and breast milk, consumed by children aged 6 to 9 months old in Chilenje

If you agree to participate in the study we will ask for your permission to do the following: -

1. We will ask for your permission to visit you at home and interview you about the types and amount foods you prepare and feed to your baby. Talking to you will greatly help us learn the accurate amount of food your child eats and will be useful in knowing more about child feeding in general.

2. In order for us to know the amount breast milk the child receives we will provide both you and your child with special water to drink on selected days over a period of two weeks. This water is not harmful and will not affect you or your child in any way as it will come out in urine. We will need to collect small amounts of urine from both you and your child on selected days over a period of two weeks. . The collected urine will only be used for the study.

3. We will ask for your permission to allow us to get small amount of blood from your baby at Chilenje clinic so that we can measure the amount of haemoglobin concentration. Measuring haemoglobin concentration in your child's blood will give us a better understanding of child health.

4. We will ask for your permission for us to measure your child's weight, length and skin folds

5. We will also ask for your permission for us to measure your weight, height and skin folds

6. We will give you a packet of food, especially prepared for the baby after all the measurements are taken

Your participation is entirely your choice. Whether you choose to participate or not, it will not affect the care you and your child receive

If you wish to know more about the study, you may ask the midwife who is seeing you or contact Mr. Victor Owino (Breast Feeding Project Office, Department of Obstetrics & Gynacology, University Teaching Hospital, Lusaka) or Dr. M. Sinkala (Lusaka Urban District Health Management Team), who are in charge of the study.

Appendix 9-3 cont.

CERTIFICATE OF INFORMED CONSENT FOR PARTICIPATION IN A STUDY TITLED 'EFFECT OF IMPROVED COMLEMENTARY FOOD ON NUTRITION OF ZAMBIAN INFANTS 6-9 MONTHS OLD.

The aim of this study is to understand the amounts of different foods, that is, the food that will be provided by the study project, foods that are traditionally used by mothers to feed children, and breast milk, consumed by children aged 6 to 9 months old in Chilenje.

I have been fully informed of this study and I am aware that should I not wish to participate in this study it will not affect the treatment of myself or my child. Equally should I consent to participation I will not be given any special services or be given payment or gifts.

I agree to allow a visit to my home for interview and that I will allow the study group to ask me the types and amounts of foods I prepare for my baby. I will allow the study group to ask me how much of each food my baby will actually eat.

I agree to drink the special water to be provided on selected days over a period of two weeks. I also agree to allow the water to be given to my child also over the same period. I agree that I will allow the study group to collect urine from my child and myself over the two weeks.

I agree to allow the study group to collect blood from my child so that they can measure haemoglobin concentration at Chilenje clinic.

I agree to allow the study group to measure my child's weight, length and skinfolds

I agree to allow the study group to measure my weight, height and skinfolds

I agree to receive the packet of food that will be provided by the study team after all the measurements

This VOization is only valid for this study.

I hereby consent to participate.

---------- ----------

Signature of participant Name (in print)

If the participant cannot write she should make a cross or a thumbprint

---------- ----------

Signature of medical officer/ research assistant Name (in print)

Date

Researcher: Victor Ochieng Owino

Address: Centre for International Child Health
Institute of Child Health
University Colle.g.e London
30 Guildford Street, WC1N 1EH London, United Kingdom

Email:- vowino@hotmail.com **. Telephone in Lusaka: 250704.**

Appendix 9-4 Randomized study household demographic data collection questionnaire

Mother-Infant ID _____

HOUSEHOLD BACKGROUND QUESTIONNAIRE

Q1. Mother's name _____

Q2. Mother's address _____

Q3. Name of index child _____

Q4. Birth weight _____ Kg

Q5. Age of index child (months)_____

Q6 Child's date of birth (dd/mm/yyyy) _____ /_____/ _____

Q7. Sex of index child (M/F) _____

Q8. Child's birth rank _____

Q9. Mother's age (yrs) _____

Q10. Occupation _____ 1= Salaried employed
2 = Self-employed
3 = Housewife
4 = Other (specify) _____ ☐

Q11. Mother's educational Level _____ 1 = Primary (1-7 Grades)
2 = Secondary (8 -12 Grades)
3 = Tertiary colle.g.es
4 = University
5 = Other specify)_____ ☐

Q12. Marital Status _____ 1 = Married
2 = Widow
3 = Divorced
4 = Single
5 = Other (specify) _____ ☐

If married: -

Q13. Husband's Age _____years

Q14. Husband's Occupation _____ 1 = Salaried employed
2 = Self-employed
3 = Unemployed
4 = Other (specify) _____ ☐

Q15. Husband's Educational Level _____ 1= Primary (1-7 years)
2 = Secondary (8 -12 years)
3 = Tertiary colle.g.es/polytechnic ☐

4 = University
5 = Other (specify) _____

Q16. Total number of persons in the household _____

Q17. Total number of children in household _____

Q18. Number of children under 3 years of age _____

Q19. Does your household have:

Electricity?	Yes/No	_____
Solar power?	Yes/No	_____
A radio?	Yes/No	_____
A television?	Yes/No	_____
Telephone or mobile phone?	Yes/No	_____
A refrigerator?	Yes/No	_____
A bicycle?	Yes/No	_____
A motor cycle?	Yes/No	_____
A car or truck (any motocar)?	Yes/No	_____

Q20. What is the material of the floor of your household house?

1 = earth/mud/dung
2 = wood planks
3 = wood tiles/parquet
4 = bricks
5 = terrazo/ceramic tiles
6 = concrete/cement
7 = carpet

[ ]

Q21. What is the main source of drinking water for members of your household?

1 = tap water
2 = borehole
3 = open well
4 = Other _____
(SPECIFY)

[ ]

Q22. Type of residence/housing? 1 = Low density
2 = Medium density
3 = High density

[ ]

Appendix 9-5 Anthropometric data record sheet for infant

Mother-

Infant ID _____

THREE-MONTH ANTHROPOMETRIC RECORD FOR THE CHILD

CHILD'S AGE (MO)	ANTHRPOMETRIC MEASURMENT										
	Weight (Kg)	Length (cm)	Head circumf. (cm)	Chest circumf. (cm)	Abdomen circumf. (cm)	Thigh circumf. (cm)	Mid Arm circumf. (cm)	Triceps Skinfold (mm)	Biceps Skinfold (mm)	Subscapular Skinfold (mm)	Suprailiac Skinfold (mm)
6 months											
Read 1											
Read 2											
Read 3											
Mean											
7 months											
Read 1											
Read 2											
Read 3											
Mean											
8 months											
Read 1											
Read 2											
Read 3											
Mean											
9 months											
Read 1											
Read 2											
Read 3											
Mean											

Appendix 9-6 Infant and maternal morbidity record questionnaires

Mother-Infant ID _____

RECORD OF CHILD MORBIDITY IN THE 3 DAYS PRECEEDING THE INTERVIEW

Visit No _____ Date_____ Time _____

Q1. Day of the week _____

Q2. Did child eat as usual?_____Yes/No

Q3. If no, why did the child not eat as usual _____

Q4. How has the child been since we last visited you (enter appropriate code in box)?

1 = generally well
2 = mild-self-limiting illness
3 = ill and treated at clinic
4 = very ill requiring antibiotics
5 = hospitalisation

Q5. Did the child experience the following in last 3 days? *(delete Yes/No appropriately)*

1. Diarrhoea (Yes/No) _____
2. Vomiting (Yes/No) _____
3. Fever (Yes/No) _____
4. Cough (Yes/No) _____
5. Wheezing (Yes/No) _____
6. Running nose (Yes/No) _____
7. Ear problems (Yes/No) _____
8. Skin problems (Yes/No) _____
9. Excessive crying (Yes/No) _____
10. Eating problems (refused food) (Yes/No) _____

Appendix 9-6 cont.

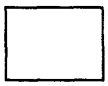
Mother-Infant ID _____

RECORD OF MATERNAL MORBIDITY IN THE LAST 3 DAYS BEFORE INTERVIEW

Visit No _____ Date_____ Time _____

Q1. How have you been since we last visited you (enter appropriate code in box)?

1 = generally well
2 = mild-self-limiting illness
3 = ill and treated at clinic
4 = very ill requiring antibiotics
5 = hospitalisation



Q2. Did you experience the following in the last 3 days? *(delete Yes/No appropriately)*

1. Diarrhoea (Yes/No) _____
2. Vomiting (Yes/No) _____
3. Fever (Yes/No) _____
4. Cough (Yes/No) _____
5. Wheezing (Yes/No) _____
6. Running nose (Yes/No) _____
7. Ear problems (Yes/No) _____
8. Skin problems (Yes/No) _____
9. Eating problems (loss of appetite) (Yes/No) _____

Name of Interviewer _____ Signature _____

Interviewer's/mother's name _____

Interviewee's signature/thumb print _____

Appendix 9-7 Protocol for deuterium dose-to-the-mother technique

Principle
The method involves the administration of a drink containing deuterium to the mother, followed by the collection of urine samples from mother and infant over subsequent days.

Anthropometry required
Maternal weight and height, infant weight and height, both at time of dosing. Any other measurements are beneficial but not required for the deuterium method itself. Re-weigh the baby on the last day of the study (day 14)

Prior to dosing
Obtain a pre-dose urine sample from both mother and infant, using the methods described below. Then, record the time and date at which the dose was given to the mother.

The deuterium dose
Each mother requires a drink containing approximately 10g of deuterium. It is not necessary to give exactly 10g, however it is necessary firstly to weigh accurately the mass of dose mixture consumed and secondly to keep a sample of each dose mixture.

Dose manufacture process:
1. weigh empty bottle
2. add approx 50 ml ordinary drinking water; tare scale
3. add approx 10 ml deuterium by syringe, using the 0.45 μm micropore filter
4. cap the bottle and shake contents gently for 1 minute to mix well, then use a fresh plastic pastette to remove a 1 ml sample and transfer this to a 2 ml micro-tube.
5. re-cap bottle, put in sealable plastic bag and weigh (WT1)
6. after dosing, reweigh empty bottle in same bag (WT2), then obviously WT2-WT1 is dose given.

Note: Ideally, use scales accurate to 2 decimal places of gram, or at least 1 decimal place. Do make sure the samples of dose are well-labelled with subject ID in a manner that will survive freezer storage.

Post-dose urine samples
These are collected according to the schedule given in the IAEA common protocol. In each case, record the time of the sample, and ensure all tubes are labelled appropriately. For infant urine samples, you will not know the exact time of the sample, so use the method described below.

Urine samples
Maternal samples can be obtained directly into a standard 30ml sterilin universal, which is then stored frozen. Label with time and date of the sample. Infant samples are most easily obtained by putting cotton wool balls, strate.g.ically placed so as to receive the urine, in the nappy, and checking re.g.ularly for moisture. Best results are obtained if the nappy is checked every 30 minutes until the sample is obtained. The time of the sample is assumed to be the midpoint between the last time the cotton wool was dry, and the first time it is damp. For example

Cotton wool put in nappy 10am
Check 10.30am? Still dry
Check 11am? Still dry
Check 11.30am? wet therefore the sample is assumed to have come at 11.15am

Transfer wet cotton wool should be immediately to a 20 ml syringe where the plunger has been pulled out, reinsert the plunger and squeeze the urine into a 2ml microtube for storage.

Note: Ensure all samples are clearly and permanently labelled with ID, time and date. Ensure there is sufficient air at the top of each tube to allow expansion of the sample during freezing. Store all dose and urine samples upright and frozen at around –15 °C until shipping for analysis.

Appendix 9-8 Record forms for deuterium dosing and two-week urine sample collection

TWO-WEEK RECORD OF STABLE WATER DOSING AND URINE COLLECTION FROM MOTHER

MEASUREMENT	DAY 0	DAY 1	DAY 4	DAY 14
Mother's weight (Kg)		NA	NA	
Mother's height (cm)		NA	NA	
Time urine taken before dosing with deuterium				
Time mother is dosed with deuterium				
Amount of deuterium dosed				
Date urine taken				
Time urine sample taken	NA			

TWO-WEEK RECORD OF URINE COLLECTION FROM CHILD

MEASUREMENT	DAY 0	DAY 1	DAY 3	DAY 4	DAY 13	DAY 14
Child's weight (Kg)		NA	NA	NA	NA	
Child's length (cm)		NA	NA	NA	NA	
Date urine taken						
Time urine sample taken						

Appendix 9-9 Sample record sheet for 2-week urine collection data for mother and infant

Mother-Infant ID	Dose taken (g)	Mother dosed Day 0		Day 0		Day 1		Day 3	Day 4		Day 13	Day 14
		Date	Time	Mother Weight, Height, Time sample got	Infant Weight Length Time sample got	Mother Time sample got	Infant Time sample got	Infant Time sample got	Mother Time sample got	Infant Time sample got	Infant Time sample got	Mother Weight, Height, Time sample got
C01	59	29.10.04	09.34	74 kg, 170.4 cm 09.32	12.8 kg, 76 cm, 09.23	09.30	09.20	10.00	10.00	10.20	10.22	73 kg 170.4 cm 09.15
C02	62	26.10.04	16.35	71 kg 162.9 cm 14.48	9.2 kg 74.5 cm 16.15	10.34	10.40	09.30	09.10	09.00	11.25	71 kg 162.9 cm 09.50
C03	62	26.10.04	13.15	52 kg 153.6 cm 12.12	10.1 kg 71 cm 13.10	-	12.40	11.15	Check on tube	Check on tube	10.30	53.5 kg 153.6 cm 10.15
C06*	58	29.10.04	14.47	51 kg 154.6 cm 14.45	8.5 kg 72 cm 14.40	Drop out	-	-	-	-	-	-
C09	62	13.11.04	10.43	52 kg 143 cm 10.40	7.4 kg 72 cm 10.25	08.19	08.15	09.30	09.20	09.15	11.30	52 kg 143 cm 10.40
C10	62	10.11.04	11.25	60 kg 160 cm 11.22	10.3 kg 73.5 cm 11.15	09.10	09.06	10.15	07.53	07.50	09.15	59 kg 160 cm 10.15
C11	60	09.11.04	10.22	66 kg 164.9 cm 10.15	11.9 kg 70.5 cm 09.56	-	-	11.40	09.55	09.47	09.40	68 kg 164.9 cm 11.00

Appendix 9-10 Excel "Solver" fitted graphs for breast milk intake and maternal body composition

Page 1 of 2

Subject [redacted]

Const errors

MOTHER'S DATA

age [redacted] Years

Weight [redacted] Kg

BABY'S DATA

sex M

age [redacted] weeks

start weight [redacted] kg

final weight [redacted] Kg

DOSE DATA

Dose (g)	[redacted] g
Edd	52.56
Et	-40
D	0.008
T	100

(1) Data for mothers saliva or milk

	time	del	Del	mean-pre-dose	sigma	Cv	del calc	chi squared
[redacted]							1879.98	
[redacted]				1634.98	0.62	0.04	1624.27	114.72
[redacted]				994.60	1.45	0.15	1016.81	493.28
[redacted]				235.71	2.73	1.16	211.14	603.49
							sum =	1211.49

(2) Data for baby's saliva or urine

	time	del	del	mean-pre-dose	sigma	Cv	Body Water	del calc	chi squared
[redacted]							6.09	0.00	
[redacted]				203.21	0.50	0.25	6.08	205.00	3.22
[redacted]				481.47	3.22	0.67	6.07	479.33	4.56
[redacted]				533.72	1.99	0.37	6.07	534.91	1.42
[redacted]				325.21	#DIV/0!	#DIV/0!	6.00	325.26	0.00
								sum =	9.20

Appendix 9-10 cont.

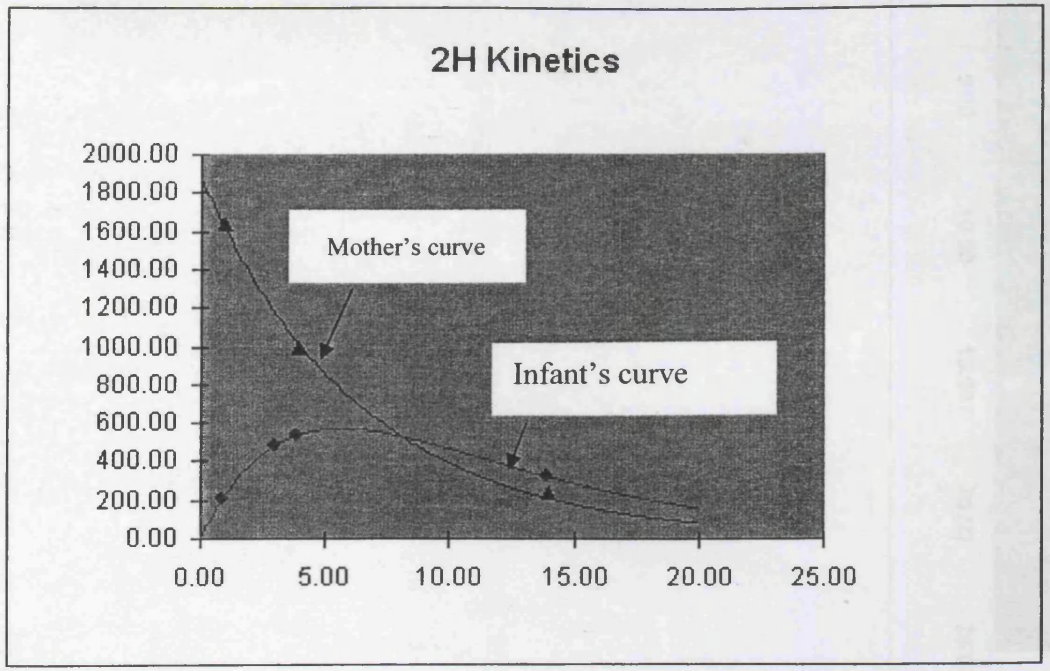
Subject *C57*

MOTHER'S COMPOSITION

D space	36.93	Kg
Lean Mass	48.64	Kg
Body fat	12.36	Kg
fat	20.27	%
wat intake	5.78	kg.day-1

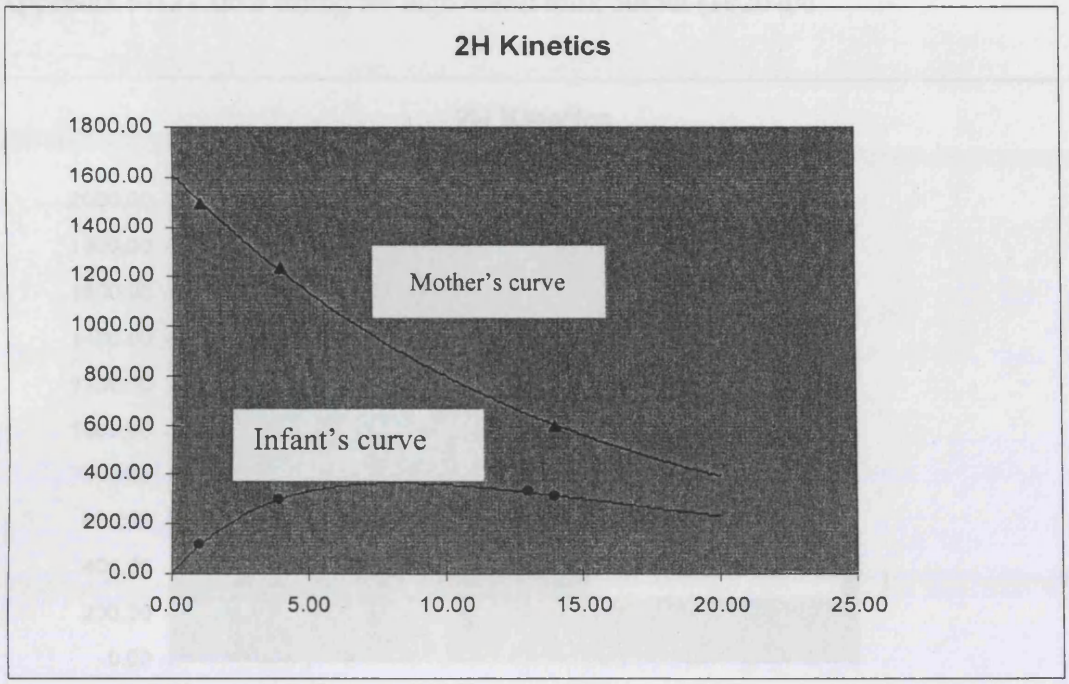
KINETIC DATA

Cm(0) =	1879.98	Del
k(mm) =	0.16	day-1
F(bb) =	1.21	day-1
F(bm) =	0.89	kg.day-1
k(bm) =	0.02	day-1

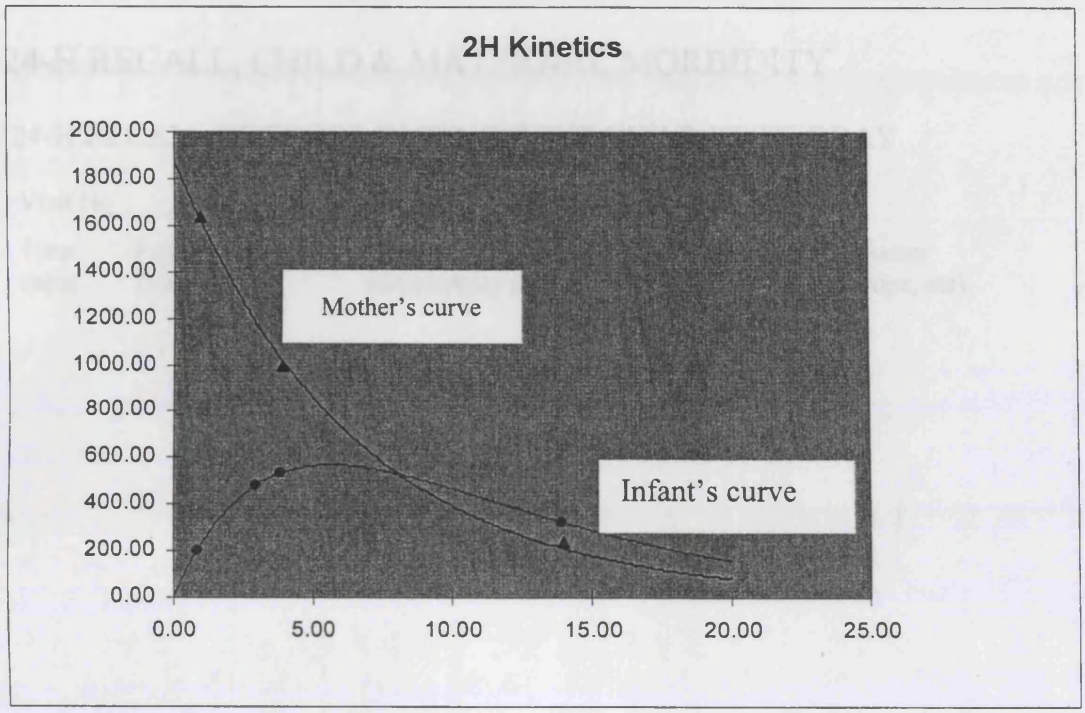


Breast milk intake (M)	1.02	kg.day-1
Water input from milk (Fm)	0.98	
Water used in growth (Fg)	-0.01	
Total water output (Fob)	1.22	
non oral water intake (Fa)	0.064	
Non-milk oral intake (Fs)	0.17	
Total error	34.94	

Appendix 9-11 Curve fitting for low breast milk intake (410 g/d)



Appendix 9-12 Curve fitting for high breast milk output (1020 g/d



Appendix 9-13 Dietary data (24-h recall) collection questionnaire

Mother-Infant ID _____

24-H RECALL, CHILD & MATERNAL MORBIDITY

24-H RECALL OF FOODS EATEN BY THE CHILD YESTERDAY

Visit No _____ Date_____ Time _____

Time eaten	Food or drink Description	Main ingredients and cooking method	Amount eaten (g, mL, cups, etc)

Is the child currently breastfed? Yes/No _____

If no, when did you last breastfeed your child (child's age-months) _____

Appendix 9-14 Nutrient composition (per 100g) on as-is-eaten basis of 67 recipes derived from 24-h recall sheets

Foodname	Foodcode	Foodgroup	Water	Energy	Prot	Fat	Carbhydr	Fibre	Phytate	VitA	VitC	VitB1	VitB2	Niacin	VitB6	Calcium	Iron	Zinc
Nshima	1001	1	72	117	3.8	2.7	20.8	2.9	82	4	0	0.01	0.05	1.4	0.08	3	1.5	0.8
Cerelac	1002	5	70	128	4.6	2.8	21.1	0.0	0	103	11	0.23	0.09	1.0	0.09	136	2.3	1.2
Bean soup	1003	7		161	2.0	15.3	7.4	1.6	11	6	8	0.11	0.12	1.9	0.28	7	1.0	0.3
E.g.g yolk	1055	6	57	285	15.5	25.0	0.2	0.0	0	874	0	0.32	0.52	4.7	0.40	140	5.5	3.8
Porridge with groundnuts	1065	2	79	107	3.3	4.7	13.1	1.7	126	20	0	0.07	0.03	1.7	0.06	7	0.8	0.5
Beef soup	1064	7		104	6.9	0.4	18.7	8.0	293	2	0	0.12	0.04	0.3	0.06	22	1.9	0.7
Porridge with cooking oil	1063	1	79	72	1.0	2.5	11.7	1.0	100	20	0	0.05	0.03	0.5	0.04	0	0.4	0.3
Nestum	1022	5	70	128	4.6	2.8	21.1	0.0	0	103	11	0.23	0.09	1.0	0.09	136	2.3	1.2
Ve.g.etable soup	1062	7		95	6.2	0.4	17.1	7.2	258	12	2	0.11	0.04	0.3	0.06	20	1.7	0.6
Chicken soup	1061	7	87	8	0.5	0.3	0.8	0.0	0	0	0	0.00	0.01	0.10	0.01	2	0.1	0.0
Porridge with soya	1028	5	79	102	5.3	2.6	15.5	2.4	192	25	4	0.11	0.07	0.8	0.05	45	1.2	0.6
Porridge with peanut butter	1029	1	79	30	1.5	2.1	6.1	0.5	55	0	0	0.03	0.02	0.7	0.04	2	0.3	0.3
Maize porridge	1004	1	79	54	1.0	0.5	11.7	1.0	100	20	0	0.05	0.03	0.5	0.04	0	0.4	0.3
Orange juice	1005	9	90	45	0.7	0.2	10.4	0.2	0	67	50	0.09	0.03	0.4	0.04	11	0.2	0.1
E.g.g fried	1013	6	80	124	7.6	9.2	2.4	0.5	0	131	4	0.06	0.31	0.2	0.09	36	0.8	0.9
Lactogen	1030	5	87	67	1.5	3.5	7.4	0.0	0	71	7	0.05	0.10	0.7	0.05	52	0.8	0.5
Vitaso	1023	5	80	75	3.7	1.9	11.0	40.0	1	174	8	0.11	0.10	1.2	0.06	166	3.5	1.0
Porridge with oil and milk	1007	1	79	110	3.1	4.7	16.0	1.0	100	39	1	0.10	0.23	0.5	0.06	75	0.5	0.5
Banana	1010	8	70	92	1.0	0.5	23.4	3.5	22	8	9	0.05	0.10	0.5	0.58	9	0.3	0.2
Nan	1038	5	70	158	3.6	8.0	17.8	0.0	0	186	16	0.11	0.24	1.6	0.12	3	1.9	1.2
Porridge with milk	1011	1	79	72	1.0	2.5	11.7	0.9	100	20	0	0.05	0.03	0.5	0.04	0	0.4	0.3
Sweet potato	1044	10	82	66	1.4	0.1	15.6	1.5	8	0	8	0.07	0.02	0.4	0.16	17	0.5	0.2
Fish soup	1006	7		101	6.8	0.4	18.3	7.6	170	11	3	0.09	0.04	0.4	0.08	31	2.0	0.8
Porridge with kapenta	1032	1	79	83	6.1	1.3	11.7	1.0	100	20	0	0.06	0.05	1.2	0.07	148	0.7	0.7
Porridge with margarine	1026	1	79	68	1.0	2.1	11.7	0.9	100	20	0	0.05	0.02	0.5	0.03	0	0.4	0.3
Custard porridge with milk	1037	5		196	7.2	11.8	15.4	5.2	341	11	143	0.13	0.06	0.1	0.11	36	2.3	0.9
Beef liver soup	1009	7		35	1.9	0.3	7.9	3.2	91	67	10	0.07	0.10	0.6	0.06	46	1.3	0.4
Chilenje Mix	1008	3	85	76	2.6	2.0	12.0	0.5	3	137	32	0.14	0.18	1.9	0.13	112	6.7	1.6
Bread	1012	1	37	274	8.8	3.0	51.9	2.7	20	0	0	0.11	0.08	0.9	0.04	37	0.5	0.8

Appendix 9-14 cont

Foodname	Foodcode	Foodgroup	Water	Energy	Prot	Fat	Carbhydr	Fibre	Phytate	VitA	VitC	VitB1	VitB2	Niacin	VitB6	Calcium	Iron	Zinc
Tea with milk	1045	9	97	40	1.9	2.3	3.0	0.0	0	33	1	0.02	0.10	0.1	0.04	75	0.1	0.3
Chilenje Mix with cooking oil	1014	3	85	86	2.6	2.0	14.5	0.5	3	162	32	0.14	0.18	1.9	0.13	112	6.7	1.6
Offals soup	1015	7		209	11.6	0.7	40.0	9.0	464	0	0	0.30	0.08	0.0	0.00	20	4.0	1.0
Irish potato	1021	10	80	73	1.1	3.1	10.6	1.0	36	15	9	0.06	0.02	0.7	0.15	4	0.3	0.2
Porridge with e.g.g	1066	1	79	81	3.3	2.3	12.1	1.0	100	44	1	0.12	0.05	0.5	0.06	8	1.0	0.5
Porridge with milk and margarine	1027	1	79	106	3.1	4.3	16.0	1.0	100	39	1	0.11	0.23	0.5	0.06	75	0.5	0.5
Chilenje Mix with milk	1016	3	85	121	5.1	4.7	17.2	0.5	3	160	33	0.21	0.42	2.0	0.16	202	6.7	1.9
Milk	1025	4	88	61	3.3	3.6	6.9	0.0	0	31.00	0.9	0.09	0.33	0.1	0.04	120	0.1	0.4
Porridge with groundnuts and milk	1067	2	79	145	5.3	7.0	17.4	1.7	126	39	1	0.13	0.24	1.8	0.09	82	0.9	0.8
Cerelac with milk	1017	5	50	237	9.2	6.3	37.6	0.0	0	182	18	0.45	0.32	0.1	0.17	280	3.8	2.1
Cassava porridge with margarine	1018	10		111	7.1	1.2	18.8	6.1	258	2	1	0.11	0.05	0.7	0.13	27	2.5	1.1
Brown mushroom soup	1019	7		115	7.1	1.2	19.8	6.1	258	2	1	0.11	0.05	0.7	0.13	27	2.5	1.1
Mango juice	1020	9	81	65	0.5	0.3	65.0	1.8	0	255	28	0.06	0.06	0.6	0.13	10	0.1	0.04
Pure joy drink	1068	9	87	51	0.0	0.0	13.3	0.0	0	0	0	0.00	0.00	0.0	0.00	5	0.1	0.1
Nutrex porridge	1036	5		101	6.8	0.4	18.2	7.6	170	11	3	0.09	0.04	0.4	0.08	31	2.0	0.8
Mashed potatoes with milk	1069	10	72	77	2.7	1.9	14.3	0.8	41	16	7	0.10	0.18	0.8	0.17	63	0.3	0.4
Custard porridge	1070	5		326	22.4	1.3	58.6	24.7	568	0	2	0.27	0.11	1.1	0.22	98	6.4	2.7
S26	1039	5		326	22.4	1.3	58.6	24.7	568	0	3	0.41	0.15	1.5	0.31	109	7.5	3.0
Maheu	1042	5	87	62	0.7	1.1	12.3	0.2	0	0	0	0.00	0.00	0.0	0.00	4	0.0	0.0
Fanta	1043	9	87	51	0.0	0.0	13.3	0.0	0	0	0	0.00	0.00	0.0	0.00	5	0.1	0.1
E.g.g boiled	1050	6	74	150	12.8	10.4	2.4	0.0	0	131	4	0.40	0.12	0.2	0.10	42.0	3.3	1.0
Rice	1071	1	75	82	1	3.1	12.2	0.4	20	0	0	0.01	0.01	0.1	0.02	0	0.1	0.2
Yorghut	1072	4	85	84	1.5	1.6	16.0	0.0	0	14	0.1	0.02	0.09	0.1	0.00	53	0.1	0.1
Cornflakes	1073	1	3	335	23.0	1.3	60.2	16.9	102	0	3	0.42	0.16	1.6	0.32	74	7.7	2.9
Vitaso with milk	1074	5	79	115	5.9	4.6	15.0	34.0	0.4	174	8	0.16	0.36	1.1	0.08	241	3.1	1.2
Potato chips	1076	10	47	250	4.0	11.0	37.0	0.7	36	0	6	0.05	0.01	0.6	0.14	2	0.2	0.1
Pork soup	1077	7		323	24.9	24.0	0.0	0.0	0	0	0	0.08	0.15	3.2	0.33	4	1.7	4.1

Appendix 9-14 cont

Foodname	Foodcode	Foodgroup	Water	Energy	Prot	Fat	Carbhydr	Fibre	Phytate	VitA	VitC	VitB1	VitB2	Niacin	VitB6	Calcium	Iron	Zinc
Pork soup	1077	7		323	24.9	24.0	0.0	0.0	0	0	0	0.08	0.15	3.2	0.33	4	1.7	4.1
Pumpkin	1078	10	90	43	0.5	0.3	4.4	1.1	6	201	5	0.05	0.01	0.4	0.04	7	0.2	0.2
Pineapple juice	1079	9	86	56	0.3	0.1	13.8	0.2	0	2	11	0.06	0.02	0.3	0.10	17	0.3	0.1
Porridge with e.g.g yolk	1080	1	79	77	2.4	2.5	11.8	1.1	121	67	0	0.08	0.07	0.9	0.08	11	1.0	0.6
Yess Drink	1081	9	87	51	0.0	0.0	13.3	0.0	0	0	0	0.00	0.00	0.0	0.00	5	0.1	0.1
Groundnut soup	1082	7		257	19.7	19.0	0.4	0.1	0	5	1	0.07	0.12	2.6	0.27	4	1.4	3.3
Rape with groundnut soup	1083	7		40	0.9	0.4	8.5	0.8	26	0	0	0.00	0.00	0.4	0.00	0	0.4	0.2
Porridge with bean soup	1084	1	79	88	2.7	3.5	12.1	2.8	120	3	1	0.06	0.03	0.4	0.05	9	0.9	0.4
Kabana drink	1085	9	87	51	0.0	0.0	13.3	0.0	0	0	0	0.00	0.00	0.0	0.00	5	0.1	0.1
Ice cream	1086	4	87	55	5.6	0.0	7.6	0.0	0	2	1	0.04	0.24	0.1	0.04	195	0.1	1.0
Purity	1087	5	5	376	19.0	3.0	73.0	0.0	0	433	35	0.55	0.40	0.0	0.30	335	19.0	44.0

Appendix 9-15 SPSS syntax command for calculation of nutrient intake

```
DO IF (FOODCODE= 1001).
COMPUTE FOODNAME="Nshima".
COMPUTE GROUP=1.
COMPUTE T_WATER=72.
COMPUTE T_ENERGY=117.
COMPUTE T_PROTEIN=3.8.
COMPUTE T_FAT=2.7.
COMPUTE T_CARBOHYDR=20.8.
COMPUTE T_FIBRE=2.9.
COMPUTE T_PHYTATE=82.
COMPUTE T_RETINOL=4.
COMPUTE T_ASCORBIC=0.
COMPUTE T_THIAMINE=0.01.
COMPUTE T_RIBOFLAV=0.05.
COMPUTE T_NIACIN=1.4.
COMPUTE T_PYRIDOXI =0.08.
COMPUTE T_CALCIUM=3.
COMPUTE T_IRON=1.5.
COMPUTE T_ZINC=0.8.
END IF.

COMPUTE WATER = T_WATER*grams/100.
COMPUTE ENERGY = T_ENERGY*grams/100.
COMPUTE PROTEIN = T_PROTEIN*grams/100.
COMPUTE FAT = T_FAT*grams/100.
COMPUTE CARBOHYDR = T_CARBOHYDR*grams/100.
COMPUTE FIBRE = T_FIBRE*grams/100.
COMPUTE PHYTATE = T_PHYTATE*grams/100.
COMPUTE RETINOL = T_RETINOL*grams/100.
COMPUTE ASCORBIC = T_ASCORBIC*grams/100.
COMPUTE THIAMINE = T_THIAMINE*grams/100.
COMPUTE RIBOFLAV = T_RIBOFLAV*grams/100.
COMPUTE NIACIN = T_NIACIN*grams/100.
COMPUTE PYRIDOXI = T_PYRIDOXI*grams/100.
COMPUTE CALCIUM = T_CALCIUM*grams/100.
COMPUTE IRON = T_IRON*grams/100.
COMPUTE ZINC = T_ZINC*grams/100.

EXECUTE.
```

Appendix 9-16 Sample SPSS database entry for calculation of infant's daily nutrient inake

Subject ID	Group	Food name	Food code	Amount eaten (g)	Time eaten
1	1	Cerelac	1002	70	B
1	1	Nshima	1001	64	L
1	1	Bean soup	1003	25	L
1	1	Orange juice	1005	50	S
1	1	Cerelac	1002	70	D
1	1	Nshima	1001	64	D
1	1	Beef liver soup	1009	25	D
3	1	Nshima	1001	64	L
3	1	Fish soup	1006	50	L
3	1	Ve.g.etable soup	1062	20	L
3	1	Chilenje Baby Mix	1008	150	D
3	1	Orange juice	1005	150	S
4	1	Chilenje Baby Mix	1008	180	B
4	1	Nshima	1001	33	L
4	1	Chicken soup	1061	50	L
4	1	Orange juice	1005	40	L
4	1	Nshima	1001	64	D
4	1	Fish soup	1006	70	D
4	1	Bread	1012	20	B
4	1	Tea with milk	1045	50	B